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# VOLATILE MONITORING AS A TECHNIQUE FOR DISEASE DETECTION IN STORED POTATOES

BY

DOUGLAS R. WATERER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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THE UNIVERSITY OF MANITOBA

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The undersigned certify that they have read a Master's thesis entitled: VOLATILE MONITORING AS A TECHNIQUE FOR DISEASE DETECTION IN POTATOES

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in partial fulfillment of the requirements for the degree of Master of Science

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External Examiner

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# VOLATILE MONITORING AS A TECHNIQUE FOR DISEASE DETECTION IN STORED POTATOES

by

Douglas R. Waterer

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

# ABSTRACT

Waterer, Douglas R., M.Sc., University of Manitoba, 1983.

Volatile Monitoring as a Technique for Disease Detection in Stored Potatoes.

Supervisor: Dr. M.K. Pritchard

The metabolic volatiles produced by healthy and E. carotovora (soft rot) infected potatoes were monitored by headspace analysis. Gas sampling syringes and a porous polymer absorbent system were used to collect the volatiles from the headspace samples prior to their analysis by gas chromatography. The effects of tuber wounding, disease variety and strain, potato variety, incubation temperature and inoculum size on the rates and patterns of volatile production were studied.

A number of quantitative and qualitative changes occurred in the volatile profiles of potatoes during the development of a soft rot infection. Elevated volatile production levels were detected very early in the development of the infection. Total volatile production increased exponentially as the disease spread. A wide range of low molecular weight organics were identified in the volatile profiles (aldehydes, ketones, and alcohols). The number of compounds detected in the volatile profiles tended to increase as the infections developed. Different relative amounts of the individual compounds were produced at various stages during the progression of the infection.

Total volatile production/unit time was the most dependable means of differentiating between healthy and diseased tubers on the basis of their volatile production characteristics. The volatile production patterns of

E. carotovora var. carotovora and E. carotovora var. atroseptica were very similar when rearing conditions were suitable for the growth of both pathogens. Different strains of the E. carotovora bacteria produced similar volatile outputs, although small differences in the growth rates of the pathogens were reflected in their volatile production. The volatile production characteristics of both healthy and E. carotovora infected 'Norland' and 'Russet Burbank' potatoes were very similar. The rate of volatile production by both diseased and healthy tubers, was strongly influenced by the incubation temperature. The range of metabolites recorded in the profiles was not affected by the temperature but the relative abundance of the various compounds was to some extent temperature dependent. Temperature related differences in the rate of development of E. carotovora var. carotovora and E. carotovora var. atroseptica infections were reflected by quantitative and qualitative differences in their volatile profiles. Increasing the inoculum size hastened disease development, changing the rate of volatile production/unit time, but not the overall volatile production pattern.

The volatile profiles of E. carotovora and C. sepedonicum (ring rot) infections differed in many respects. Total volatile production/unit time was much lower in the C. sepedonicum infection. The C. sepedonicum profiles lacked a number of the compounds isolated in the E. carotovora profiles but the C. sepedonicum infections featured one unique diagnostic compound. The relative importance of the individual peaks in the C. sepedonicum profiles were clearly different from the same peaks in the E. carotovora profiles.

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DEDICATED TO:

1) CORNELL'S LAW:

Under the most rigorously controlled conditions of pressure, temperature volume, humidity, and other variables the organism will still do exactly as it pleases.

2) SMITH'S LAW:

There's never time to do it right, but there's always time to do it over.

TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

# LIST OF FIGURES

# INTRODUCTION

The majority of North American potatoes are stored in large controlled atmosphere storages. This large scale centralized storage strategy provides the efficiency of handling and longterm storage vital to the industry. However, the trend towards larger storages and longer storage periods has led to a number of management problems. Besides the increased potential for losses due to damage, respiration, evaporation, and sprouting, the problem of disease in stored potatoes becomes more serious as the storage period is extended. Not only do the potatoes become more susceptible to disease with longer storage, but the longer storage periods also increase the period available for the development and spread of any disease.

Adequate monitoring of the quality and disease status of the crop during storage has been found to be a vital measure in the efficient longterm storage of potatoes. However, the monitoring systems presently available for checking the status of large potato storages have proven to be inadequate for the early detection of disease. Consequently, bacterial disease, such as bacterial soft rot (Erwinia carotovora), have become an increasingly serious threat because of their ability to spread rapidly from small non-detectable infection loci.

Monitoring of the volatile organic metabolites produced by a host-pathogen interaction has been proposed as a remote disease monitoring technique for agricultural storages. By monitoring the quantitative and qualitative changes in the volatile profile of the storage atmosphere during the storage period, detection and identification of developing disease problems

may be possible. Information on the size, developmental status, and location of the disease problem could potentially be provided by volatile production patterns.

The primary purpose of this research was to investigate the changes which occur in the volatile profile of potatoes during a disease infection. Specifically, the objectives of the project were to:

1) Isolate and identify some of the significant metabolic volatiles of both healthy tubers and those infected with E. carotovora bacteria.

2) Assess the quantitative and qualitative changes in the volatile profile which occur during the progression of the disease in order to establish diagnostic factors which might aid in the early detection and identification of the disease.

3) Determine if variables such as inoculum size, incubation temperature, cultivar of potato, or the strain of pathogen have a significant impact on the volatile profile.

Some of the available atmospheric volatile sampling methodologies were also evaluated relative to the problem of monitoring disease-induced volatile metabolites in agricultural storages.

# REVIEW OF LITERATURE

## Introduction

The potato (Solanum tuberosum L.) is noted for its susceptibility to fungal and bacterial diseases during storage. Total wastage and storage losses resulting from disease may range from 5% to as high as 25% of the stored crop depending upon disease levels and storage conditions (Nash 1978).

In North America, bacterial soft rot (Erwinia carotovora) is the most potentially destructive of the storage diseases (Nash 1978; Perombelon and Kelman 1980). Approximately 180,000 - 300,000 tonnes of potatoes are lost annually in the United States to bacterial soft rot decay during storage (Varns 1981). Under suitable conditions the disease may cause extensive rotting of tubers within 2 to 5 days of infection (Perombelon 1969; Varns and Glynn 1979). The disease may also spread rapidly from tuber to tuber (Hooker 1978), and, if not detected and arrested, the incipient infection may envelop the entire bin.

In commercial storages, the key to the control of losses to bacterial soft rot is early detection of the problem. Localized disease outbreaks may be controlled relatively easily if detected in the early stages (Roberts et al. 1976; Varns and Schaper 1981). Predictably, eventual losses are directly related to the duration of disease development prior to the preventative modification of the storage environment (Roberts et al. 1976; Sparks 1979).

## Storage Monitoring

Visual monitoring. In the past, visual inspections of the potato storages were usually sufficient to ascertain the quality and disease status of the stored crop (Nash 1978; Sparks 1979). The growers looked for signs of obvious spoilage on the surface of the pile and checked the floor for signs of seepage (Roberts et al. 1976; Nash 1978). In today's massive commercial storages the visible surface of the pile can provide only limited information on the status of the entire mass (Varns and Glynn 1979).

The size of present storages has necessitated the utilization of remote monitoring systems capable of evaluating conditions deep within the potato pile (Schaper and Varns 1978; Varns and Glynn 1979; Varns and Schaper 1981). In general, these systems rely on the detection of some by-product of the physiochemical processes involved in any type of spoilage, disease or insect infestation (Lee et al. 1973; Varns and Glynn 1979).

Temperature monitoring. The goal of monitoring the temperature of agricultural storages is the detection of the heat produced by the metabolic processes of any developing pathogen, as well as the heat evolved in the disease resistance reactions of the stored crop (Roberts et al. 1976). In unventilated storages (i.e. grain bins) air movement is limited and thermal diffusion and dissipation through the stored crop is slow (Sinha and Wallace 1965). Consequently, the heat produced by any form of spoilage or disease remains relatively localized and may be detected by suitable temperature monitoring systems (Muir 1974). With potatoes, the "normal" metabolic heat output at recommended storage temperatures is such that the product must be ventilated throughout the storage period in order to maintain optimum storage conditions (Roberts et al. 1976; Nash 1978; Sparks 1979). Consequently,

the metabolic heat produced by any disease is rapidly dissipated. Therefore, temperature monitoring systems in potato storages are normally only useful for detecting large-scale temperature changes and early detection of small pockets of decay is unlikely (Varns and Schaper 1981). Despite the limitations of the system, temperature monitoring via thermocouples in the load is the most common and often the only systematic technique used to monitor the disease status of large-scale potato storages (Roberts et al. 1976; Anon 1977; Nash 1978).

Volatile monitoring. The normal physiological processes of all organisms involve a wide range of metabolic intermediates and by-products that are volatile (significant degree of vaporization at standard temperature and pressures) (Zlatkis et al. 1971; 1973a, b). The onset of any disease causes significant alterations in both the metabolic rate and physiological processes of the infected organism (Zlatkis et al. 1973; Varns and Glynn 1979). These alterations are reflected by significant changes in the volatile metabolite profile of the diseased organism (Zlatkis et al. 1973; Varns and Glynn 1979). Zlatkis and his associates (Zlatkis et al. 1971 ; 1973a, b) were the first to propose that diseases could be detected by monitoring volatile metabolite levels. They were eventually able to detect various human metabolic disfunctions (kidney disease, diabetes) through the analysis of the volatiles present in the patients' breath and urine (Zlatkis et al. 1971; 1973a, b).

The volatile profiles of most agricultural crops have been extensively investigated (Self 1967; Rasekh and Kramer 1971; Dravneiks et al. 1973; Salunkke and Do 1976), but these profiles were for healthy rather than diseased material. Information on the volatile profiles of healthy agricultural crops during normal storage is limited (Hougen et al. 1971; Rasekh and Kramer 1971; Meigh et al. 1973; Richard-Moulard et al. 1976; Abramson et al.

1980). Microbiological research has produced considerable information on the volatile metabolites evolved by various micro-organisms, including numerous strains known to be stored product pathogens (Lamanna and Malette 1959; Henis et al. 1966; Adamson et al. 1974). Kaminski and co-workers (1972 and 1973) were able to identify a number of volatile odor components produced by the fungi (Aspergillus flavus, Penicillium sp. and Cephalosporium) that infect stored cereal crops.

Lee et al. (1973) applied the available volatile monitoring methodology as a non-destructive technique for the detection of very low levels of mycotoxin-elaborating Aspergillus flavus and A. parasiticus in peanut stocks. Eventually they were able to detect the mold development simply by measuring the increased volatile production of the contaminated stock relative to healthy nuts.

Richard-Moulard et al. (1976) conducted sequential studies of the changes in the volatile profile during the storage of corn in conditions favourable to the development of microflora. They observed profound quantitative and qualitative changes in the volatile profile with time, particularly with the onset of spoilage.

Abramson et al. (1980) monitored the levels of several known fungal metabolites over a 20 week period in a farm-scale wheat storage bin. They were able to correlate changes in the volatile profile with a number of storage quality parameters including fungal population levels.

Varns and Glynn (1979) have conducted the only study on storage volatile monitoring for disease detection in potatoes. In laboratory experiments, they monitored the volatile dynamics of potatoes inoculated with soft-rot bacteria (Erwinia carotovora var. atroseptica). They noted significant quantitative and qualitative changes in the volatile profile through the

progressive stages of the infection. The concentrations of several compounds in the diseased profile were 15 to 1500 times greater than those observed in the healthy volatile profiles. During the development of the infection the ratios of acetone and ethanol relative to 2-butanone and acetoin changed. Further, a number of compounds were detected in the volatile profile of diseased tubers that were not apparent in the healthy tuber profiles. Three compounds, ethanol, acetone, and 2-butanone, were identified as having particular potential as indicators of bacterial soft rot.

Varns and Glynn (1979) also conducted tests in 500 to 600 tonne capacity commercial storages in which they compared the volatile characteristics of storages known to be infected with E. carotovora soft rot and Fusarium spp. dry rot with the output from apparently healthy bins. The levels of ethanol, acetone, and 2-butanone detected in the diseased bins were significantly greater than in the healthy storages. It was speculated that the nature of the changes in the volatile profile could potentially provide information on the pathogen involved in an infection, the size of the trouble spot and the stage of development of the infection (Varns and Glynn 1979; Varns and Schaper 1981).

Volatile Monitoring Methodology

Wyllie et al. (1978) and Varns and Glynn (1979) concluded that the concept of volatile monitoring as a disease detection technique hinges on the utilization of an analytical system capable of consistently and objectively making rapid, comparative quantitative and qualitative assessments of the volatile profile of the storage atmosphere over the entire storage period. Varns and Glynn (1979) state that an experienced potato storage manager may gain considerable information on the physiological and disease status

of a storage from the odor eminating from the pile. Many management decisions continue to be based on this human form of volatile monitoring (Varns and Glynn 1979). Unfortunately, the human nose is neither extremely accurate nor dependable (Heath 1978). Additionally, many potentially important volatile organics are essentially odorless to humans (Abramson et al. 1980).

Volatile monitoring as a technique for disease detection is based on the concept of the storage as an equilibrium system. The atmosphere above the stored product (the headspace) is assumed to be in equilibrium with the product. Consequently, a sample of the headspace atmosphere should both quantitatively and qualitatively represent the volatile characteristics of the stored crop (Wyllie et al. 1978).

Headspace analysis techniques have been utilized in an extremely diverse array of research endeavours (see Charlambous 1978 for review). Many of the operational and methodological problems and limitations that have been encountered in this previous research must be considered in the application of headspace analysis techniques to the problem of disease detection by volatile monitoring (Varns and Glynn 1979).

Sample collection. In most headspace sampling projects, sample collection is a serious problem since the volatiles of interest are generally found at extremely low concentrations in the sample atmosphere (Wyllie et al. 1978; Boyko et al. 1978; MacKay and Hussein 1978). A number of systems have been devised for the collection of headspace samples of sufficient concentration for gas chromatographic analysis (Nawar and Ferguson 1962; Flath et al. 1969; Clark and Crunin 1975; Dupuy et al. 1978). In direct sampling, a quantity of headspace atmosphere is introduced directly into the gas chromatograph. The largest headspace sample which can be practically introduced directly into a gas chromatograph is about 10 $cm^3$ (Wyllie et al. 1978; MacKay and

Hussein 1978). There is no concentration step in this procedure, consequently, only compounds with a high vapor pressure or a high concentration in the sample are detectable with this sampling technique ( Levin and Ikeda 1968; Bertucioli and Montedoro 1974). The injection of larger samples is not feasible as this leads to excessively slow and difficult sample injections as well as to problems with peak broadening, non-reproducible retention times, and column pressure imbalances (Jennings et al. 1974; Wyllie et al. 1978).

If the volatile compounds of interest are not sufficiently concentrated in a direct headspace sample, a headspace concentration procedure may be used to increase the detectability of the volatiles (Boyko et al. 1978; Wyllie et al. 1978).

In cryogenic sampling and concentration, the headspace sample gas is passed through a trap which is cooled to below the dew point of the sample volatiles (Bertsh et al. 1974; Wyllie et al. 1978). The volatiles are collected and concentrated in the condensation trap (Bertsh et al. 1974). However, since the cooling zone is generally held at temperatures well below $0^{o}$ C, water vapor in the sample air freezes out along with the volatile organics (Bertsh et al. 1974; Vanhaelan et al. 1977). This water dilutes the volatile sample and detrimentally affects column performance if introduced into the chromatograph (Wyllie et al. 1978).

Charcoal can be used to remove organic volatiles from the air (Mieure and Dietrich 1973). However, sample recoveries from the charcoal are difficult (Bertsh et al. 1974; Barnes et al. 1981).

There are many highly porous, thermally stable polymer beads (polyethylene/polystyrene co-polymers) that have been developed as gas chromatography column materials (Chromosorb Century series from Johns Mansville, Porapak series from Waters Associates and Tenax G.C. from Applied Chemical Services) which

were also suitable for use as trapping agents in headspace sampling experiments (Bertsh et al. 1974; Jennings et al. 1974; Barnes et al. 1981). These polymers are capable of efficient, non-specific concentration of volatiles from large volumes of headspace (Mieure and Dietrich 1973; Murray 1977). The headspace sample being moved through the trap acts as both the carrier gas and a means of continuous sample introduction (Wyllie et al. 1978). As the gas sample moves through the trap the volatiles are removed by capillary condensation on the highly porous beads (Bertsh et al. 1974). At any given temperature, each compound in the headspace sample has a specific degree of affinity for the absorbent (Butler and Burke 1976). Once the trap reaches its retention capacity for any given compound, any further addition of that compound to the trapping column is balanced by the loss of an equal number of molecules of that compound further along the column (Mieure and Dietrich 1973). The retention volume of the earliest eluting compound of interest (lowest absorbent affinity) defines the maximum volume of headspace air which can be sampled quantitatively (Mieure and Dietrich 1973; Wyllie et al. 1978).

As the porous polymers are non-polar and highly hydrophobic, the problem of excessive water loading during the sampling of biological systems is eliminated (Vanhaelen et al. 1977; Mieure and Dietrich 1973). The relatively small chemically active surface area of the porous polymer reduces the problems of chemical modification and irreversible compound binding that are encountered with charcoal traps (Clark and Cronin 1975; Wyllie et al. 1978). The desorption temperatures necessary to completely purge the volatiles from the polymer traps are generally low enough ( $< 200^{o}$ C) that pyrolytic alteration of the volatile sample during the desorption procedure is not a problem (Bertsh et al. 1974; Clark and Cronin 1975).

In order to obtain quantitatively and qualitatively valid and representative data in any headspace analysis project, the vapor pressure, polarity, concentration, structural nature, and boiling point range of the volatile components of interest in the headspace sample must be taken into consideration during the development of the sampling procedure (Kuo et al. 1977; Wyllie et al. 1978; Bertsh et al. 1974; Kolb 1976; Bertuccioli and Montedoro 1974). The operating characteristics of the trapping polymer (chemical affinity, polarity, active area/gram) the structure and capacity of trap, and the sampling strategy (sample size and rate) must also suit the headspace environment of interest (Murray 1977; Butler and Burke 1976; Barnes et al. 1981).

## Soft Rot as a Pathogen in Potato Storages

A wide range of bacteria including pectolytic representatives of Erwinia, Pseudomonas, Clostridium, Flavobacterium, Xanthomonas, and Bacillus are capable of producing soft rot in potatoes (Hayward 1974; Hooker 1979; Perombelon and Kelman 1980). However, the soft rot erwinias are the most important in terms of their prevalence, virulence, and economic impact in stored potatoes (Nash 1978; Perombelon and Kelman 1980; Varns and Schaper 1981).

Taxonomy. Erwinia bacteria are gram negative, rod-shaped, non-spore forming, faculative anaerobes (Bergey 1974). Species of the genus that belong to the 'carotovora' (carrot devourer) group are generally regarded as the soft rot erwinia types, i.e. E. carotovora (Jones) Bergey et al. var. carotovora (Jones) Dye and E. carotovora (Jones) Bergey et al. var. atroseptica (Van Hall).

The primary characteristic which distinguishes the soft rot erwinias from the other members of the genus is their ability to produce and secrete large quantities of tissue macerating enzymes (Starr and Chatterjee 1972) which enable the bacteria to attack a wide range of plant species, both in the field and after harvest (Agrios 1967).

Differentiation of E. carotovora varieties. E. carotovora var. atroseptica (ECA) and E. carotovora var. carotovora (ECC) are physiochemically very similar (Perombelon and Kelman 1980). The taxonomic differentiation of the varieties is based primarily upon biochemical reactions (Graham 1972). E. carotovora var. carotovora has a minimum temperature requirement of 6° C for growth while optimal growth occurs at 28 to 30° C and the maximum limit for growth is 37 to 42° C (Graham 1972; Perombelon 1974). The minimum,

optimum, and maximum for growth of ECA are somewhat lower, at 3° C, 27° C, and 35° C, respectively (Perombelon and Kelman 1980).

E. carotovora var. atroseptica is generally restricted to temperate regions (Perombelon 1973; 1974; Burr and Schroth 1977) and its host range is limited to cool season crops (Stranghellini and Meneley 1975). By contrast, ECC with its higher temperature tolerances, occurs in both tropical and temperate regions and is pathogenic to a wider range of plants (Graham 1964; 1974). In suitable regions, both varieties are commonly isolated from one infected plant (DeBoer and Kelman 1975).

Etiology and histopathology. When the soft rot erwinias attack a stored product, the symptomatology is similar irrespective of the host species or the variety of E. carotovora involved (Perombelon and Kelman 1980). In potatoes, the lenticels are the primary site of infection. However, the disease may be initiated wherever the pathogen has penetrated the tuber (Hooker 1978; Perombelon and Kelman 1980). The bacteria generally reside and multiply within the intercellular spaces of the infected tissues (Starr and Chatterjee 1972). There they secrete a battery of pectolytic enzymes which attack the pectic substances that act as structural cement in the cell wall matrix and middle lamellate of the host tissues (Goto and Okabe 1958; Starr 1959; Graham 1964). Smaller amounts of cellulytic enzymes are also released, resulting in localized destruction of the cell walls (Hooker 1978). Deprived of structural support the host tissues lose their physical integrity (Hooker 1978). The protoplast of the attacked cells collapses and dies, releasing the cellular contents into the intercellular spaces (Starr and Chatterjee 1972). This cellular sap provides nourishment and a means of dissemination for both the pathogen and the wide range of secondary saprophytes (Clostridia, E. coli and Fusaria) which rapidly invade the infected

area (Meneley and Stranghellini 1972; Hayward 1974). Externally, the infection appears as sunken, cream to brown, water-soaked areas (Hooker 1981). As the disease advances, the diseased tuber begins to crack, oozing a highly infectious bacterial slime (Hooker 1978). Eventually, only the corky epidermis of the tuber remains untouched by the pathogen.

Soft rot in storage. Although the potato may be attacked by soft rot bacteria in the field, the development of the pathogen during the storage period is often the most significant form of loss to the soft rot erwinias (Varns and Glynn 1979; Perombelon and Kelman 1980). In a study of commercial storages in Manitoba, Poff (1979) isolated E. carotovora from 59% of all tubers tested. Perembelon (1974), working in Scotland, and Nelson (1978) and Aleck and Harrison (1978), in the United States, found ECA to be the dominant soft rot erwinia in the tubers tested in their regions. E. carotovora var. carotovora was found to be more prevalent in tubers taken from storages in Romania (Lazur and Bucur 1964) and Manitoba (Poff 1979).

Initial inoculum levels may vary from 0 to $10^5$ bacteria per tuber in surface infections and 0 to $10^2$ bacteria per lenticel (Perembelon and Kelman 1979). The subsequent survival of the bacteria during storage depends upon their location on the tuber (Hayward 1974). Surface populations of soft rot bacteria decline drastically during storage, while the bacteria sheltered within cracks and in the lenticels are virtually unaffected.

Because of the disease kinetics of bacterial soft rot in potato storages, the disease potential of the crop during storage is not predictable, nor is it necessarily directly determined by the incidence of contamination at the onset of the storage period (Varns and Glynn 1979). As the soft rot infection will spread from tuber to tuber in suitable conditions, the entire storage may eventually be threatened by an infection originating from a single

point of decay (Nash 1978). Alternatively, as the bacteria will only develop under suitable storage conditions a high incidence of tuber contamination need not inevitably lead to excessive spoilage losses (Roberts et al. 1976; Nash 1978).

Disease dynamics in storage. Several factors in the storage environment are known to influence the development of a bacterial soft rot infection (Perembelon and Kelman 1980). E. carotovora infections in potatoes progress more rapidly under reduced oxygen concentrations, despite the fact that, in vitro, the soft rot erwinias grow better under aerobic than anaerobic conditions (Lund and Nichols 1970; DeBoer and Kelman 1978). There may be a reduction in the disease resistance of the tubers with declining oxygen concentration (Lichovitch et al. 1967). Under aerobic conditions, rotting will occur only if there are sufficient bacteria ($10^6$ to $10^7$) to produce anaerobiosis on a micro-scale at the infection site (DeBoer and Kelman 1978). When the host tissues are oxygen deficient, only a few dozen bacteria are required to elicit decay (Perombelon and Lowe 1975).

Water films on stored produce increase decay and inhibit the potatoes' resistance systems by interfering with the diffusion of oxygen into the tuber (Burton and Wigginton 1970). A water film also facilitates the movement of bacteria to previously uninfected sites (Perembelon and Kelman 1980).

Temperature is the primary environmental factor affecting the development of soft rot in potato storages. At normal storage temperatures (5 to 9° C), bacterial growth is limited and the resistance responses of the potatoes are normally sufficient to further retard the onset of any decay (Perombelon and Kelman 1980). At these low storage temperatures, some form of external interference, such as the development of free water on the tubers, must take place before rotting will occur (Perombelon and Kelman 1980). The physio-

logical processes involved in wound healing and disease resistance in the potato reach a maximum at about 20° C and at temperatures above this point disease development is very rapid (Perombelon and Lowe 1975).

Disease characteristics. Wet conditions at harvest increase the susceptibility of potatoes in storage to soft rot (Hooker 1978; Nash 1978). In very wet years the tubers may already exhibit some degree of rot at harvest (Nash 1978). Potatoes harvested at soil temperatures above 23° C tend to have severe disease problems because of a high field heat content going into storage (Hooker 1978).

Before a storage is cooled to the 'safe' longterm storage temperatures, the potatoes are held at 15 to 18° C for 10 to 14 days to allow for wound healing (Sparks 1979). These warm conditions may trigger bacterial decay in the harvest damaged tubers (Nash 1978; Hooker 1978).

Maintenance of the recommended 90 to 95% R.H. in the storage without the development of condensation on either the potatoes or the walls of the storage (which will drip on the load) is difficult (Nash 1974; Roberts et al. 1976). In storage, liquid from frost damaged or diseased tubers may drip on sound but E. carotovora contaminated stock creating conditions favorable for decay (Perombelon and Kelman 1980).

The onset of disease can occur almost simultaneously throughout the storage (Nash 1978). This type of outbreak usually occurs early in the storage period and stems from environmental conditions prior to or during harvest that are favorable for the development of the pathogen population (i.e., warm, wet soil) (Nash 1978). However, the most common type of storage decay is the development of localized areas of infection within the storage as a result of disease and storage conditions combining in one area to produce an environment suitable for the onset of decay (Roberts et al. 1976;

Perombelon and Kelman 1980).

Management of disease in storage. When unfavorable harvest conditions trigger a widespread outbreak of disease in the newly harvested tubers, the temperature of the storage is decreased as rapidly as possible (Nash 1978; Sparks 1979). Air flow rates are increased to dry and cool the tubers (Roberts et al. 1976). These procedures slow the development of the infection, but without having undergone adequate wound healing, the storage potential of the potatoes is limited (Roberts et al. 1976).

In cases where pockets of decay are produced by localized unfavorable storage conditions the focus of decay is removed, if possible, to prevent any further spread of the disease (Sparks 1979). As an insurance measure airflow rates through the remaining tubers are increased to dry any surface moisture or leakage (Sparks 1979).

# MATERIALS AND METHODS

## General Procedures

### Tuber Preparation

The potatoes (1979 and 1980 crops) used in all experiments were stored (1 to 8 months) in darkness at 10° C and 90% relative humidity prior to use.

Sound tubers weighing 200 ± 5 g were selected for all experiments. The tubers were washed, then surface sterilized by soaking for 30 min in a 1% solution of a quaternary ammonia disinfectant[1]. The tubers were subsequently rinsed with tap water, then immersed for 30 min in distilled water. This final immersion was necessary to hydrate the outer cell layer of the potato. Once hydrated, the tuber surface is more amenable to the survival and establishment of bacteria.

### Soft Rot Inoculation

Isolates of E. carotovora var. carotovora (ECC) and E. carotovora var. atroseptica (ECA) were obtained from the collection maintained by the Plant Pathology Section of the Manitoba Department of Agriculture. The bacteria were maintained on nutrient agar slants at 2° C until the time of use. Inoculum was prepared by inoculating 50 ml swirl cultures of either standard potato dextrose[2] or nutrient broth[3]. After 72 h of incubation at 21 ± 2° C

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[1]Teramine - West Chemical Products; Montreal, Quebec, Canada.

[2]Difco Manual.

[3]Laboratory Guide for the Identification of Plant Pathogenic Bacteria. ed. N.W. Schaad.

the bacteria population was approximately $4.0 \times 10^7$ colony forming bodies/ml.

An aliquot of undiluted culture was introduced into each tuber via a syringe equipped with a sterile 3.8 cm No. 186 hollow needle. Unless otherwise specified each tuber was punctured at 25 points during inoculation. The total inoculum volume was 1 ml/tuber unless otherwise specified. Tubers which had been wounded but not inoculated were used as the reference control for volatile production.

Jar Experiments (Experiments 1 to 3)

Sterile, volatile free, 946 ml glass Gem jars were used as the incubation and headspace confinement chambers in this series of tests. One inoculated tuber was placed into each jar. Although the jars featured a screw-top lid with a rubber sealing gasket, it was necessary to wrap the top of the jar with parafilm to ensure a gas-tight seal. The lid of the jar was fitted with a rubber septum to permit gas sampling.

In all jar experiments, ambient air was used as the storage environment in the jars. The jars were stored in darkness at $21 \pm 2^{\circ}$ C.

The enclosed potatoes were sampled for volatile production every 24 h using a 10 $cm^3$ capacity gas-tight syringe[4]. Before the gas sample was taken, the syringe was filled and emptied several times with the air from the jar to be sampled. This process allowed the sample volatiles to equilibrate with the syringe materials and also dispersed any volatile gradients that may have existed within the jar. The 10 $cm^3$ gas samples were introduced onto the gas chromatograph column by direct injection. However, prior to its introduction into the gas chromatograph, the sample was compressed to 2 $cm^3$, through the

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[4]Pressure Lokk Syringe - Precision Sampling Co.; Baton Rouge, Lou., U.S.A.

exertion of pressure on the syringe plunger while the syringe outlet valve remained closed. This pressurization of the sample prior to injection facilitated rapid, reproducible sample introduction.

Immediately after sampling, the rubber septa were removed from the jar lids and the tubers were misted with sterile, distilled water in order to maintain a suitable habitat for the growth of the pathogen. The jars were then thoroughly flushed with ambient air prior to being resealed. This air constituted the storage environment for the next sampling period.

Two experiments were conducted using jars as the incubation and headspace confinement chamber. In the first experiment 'Russet Burbank' tubers were treated with a mixed inoculum which was prepared by mixing equal volumes of mature E. carotovora var. carotovora and E. carotovora var. atroseptica swirl cultures. This experiment was sampled as a completely randomized design, with nine replicates for the inoculated treatments and three replicates for the control. In the second experiment, the disease organisms (ECC or ECA) were introduced into 'Russet Burbank' and 'Norland' tubers. The experiment was sampled as a completely randomized design with three replicates/treatment. This experiment was conducted twice (Experiments 2 and 3).

Bag Experiments (Experiments 4 to 10)

Sterile, airtight (3.2 L capacity, 0.08 mm thickness) ziplock bags[5] fitted with a single gas outlet were used as the incubation environment and headspace confinement chamber in these tests. Unless otherwise specified, the tubers to be tested were prepared and inoculated in the previously described manner. One kilogram of inoculated tubers was placed in each bag.

[5] Polyethylene/Saran copolymer. Dow Chemical Canada, Toronto, Canada.

With the potatoes in place, the sealed bags contained 2.5 L of air. Room air, which had been filtered through a cotton-wool plug to reduce bacterial contamination, was used as the headspace environment.

The bags were stored in darkness throughout the test period. Unless otherwise specified, the storage temperature was 21 $\pm$ 2$^{o}$ C. Volatile samples were taken from the bags at 24 h intervals in a manner that will be described in the "Bag Sampling Procedure" section. The bags were sampled in a completely randomized manner in all experiments. Immediately after each sampling the bags were completely evacuated. The potatoes were then misted with sterile, distilled water to maintain a surface moisture film. The new storage atmosphere was then metered into the bags.

Experiment 4: Volatile profiles of diseased and healthy tubers. To compare the volatile profiles of diseased versus healthy tubers a mixture of E. carotovora var. carotovora and E. carotovora var. atroseptica was used to infect the potatoes. This mixture of pathogen types represents the expected inoculum situation in commercial storages (Perombelon and Kelman 1980).

E. carotovora var. carotovora (ECC) and E. carotovora var. atroseptica (ECA) swirl cultures were prepared as previously described. A mixed inoculum (EMX) was prepared by combining equal volumes of the two swirl culture. 'Russet Burbank' tubers were used in this experiment. Nine replicates of the "diseased" treatment (EMX) and two replicates of the "wounded control" (CO) treatment were prepared.

The experiment ran for 5 days. Beyond this time, it was not possible to sample the "diseased" treatments due to disintegration of the tubers.

Experiment 5: Volatile profiles of different varieties of E. carotovora. This experiment was designed to test the difference between the volatile

profiles of ECC and ECA infections. Additionally, the volatile profiles of diseased versus healthy tubers and the effect of wounding on volatile production were investigated.

'Russet Burbank' tubers were inoculated with either ECA or ECC in the "diseased" treatments. "Wounded controls" were prepared as before. Additionally, non-wounded tubers were used in a "healthy control" treatment. Five ECC, five ECA, two wounded control (CO), and two healthy control (CX) treatments were prepared.

This experiment was conducted twice.

Experiment 6: Volatile profiles of different strains of E. carotovora. This experiment was designed to determine the volatile production of different strains of the two E. carotovora varieties. Additionally, the volatile profiles of diseased versus healthy tubers and ECA versus ECC were investigated in this test. This experiment was conducted twice. In the second trial, the effect of using different cultivars of potatoes as the disease host was also investigated.

Swirl cultures of two strains of E. carotovora var. atroseptica (ECA 1 = previously utilized strain and ECA 2)[6] and three strains of E. carotovora var. carotovora (ECC 1 = previously utilized strain, ECA 2, and ECA 3)[6] were prepared in the previously described manner. The first time this experiment was conducted, only 'Russet Burbank' (R) tubers were tested. Three replicates of each treatment were prepared. The second time this experiment was conducted, both 'Norland' (N) and 'Russet Burbank' (R) were tested. Two replicates were prepared for each treatment.

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[6]Provided by Plant Pathology Section, Manitoba Department of Agriculture.

Experiment 7: Effect of incubation temperature on volatile profile of diseased potatoes. This experiment was designed to test the volatile production of diseased versus healthy tubers at a range of incubation temperatures. The goal was to determine the effect of temperature on the combined production and diffusion of the metabolic volatiles from the point of production.

An E. carotovora var. carotovora/E. carotovora var. atroseptica mixed inoculum (EMX) was prepared in the previously described manner. 'Russet Burbank' tubers were treated to produce 12 "diseased" and six "wounded control" treatments. The tubers were incubated at 21.5, 9.8, and 4.1° C, with four "diseased" treatments and two "wounded controls" at each temperature.

The experiment was conducted twice.

Experiment 8: Effect of incubation temperature on volatile profiles of potatoes infected with E. carotovora var. carotovora or E. carotovora var. atroseptica. As in Experiment 7, this test was designed to investigate volatile production patterns at different incubation temperatures. However, the E. carotovora varieties (ECA and ECC) were tested separately in order to ascertain whether the temperature-related difference in growth of the varieties (Perombelon and Kelman 1980) would be reflected in the volatile output.

Inocula for ECA and ECC were prepared in the previously described manner. The treatments were incubated at 21.5 and 9.8° C (three replicates for each disease variety and two wounded controls/temperature).

This experiment was conducted twice.

Experiment 9: Effect of inoculum size on volatile production and temperature effects on trap performance. Besides testing the effect of inoculum size on volatile production, the effect of temperature on trap performance was also tested.

An E. carotovora var. carotovora/E. carotovora var. atroseptica mixed inoculum (EMX) was prepared in the previously described manner. A "small-inoculum diseased" treatment (SMX) was prepared by inoculating each tuber with 1 ml of the mixed inoculum distributed over 25 puncture points. The large-inoculum "diseased" treatments (LMX) were prepared by inoculating the tubers with 2 ml of the mixed inoculum distributed over 50 puncture points. The corresponding "wounded controls" were SCO (25 punctures) and LCO (50 punctures). In total, 16 treatment bags were prepared with four replicates for each treatment (SMX, LMX, SCO, LCO).

During the sampling of two replicates for each treatment, the collection traps were maintained at 1° C, in an ice-water bath. For the other two replicates, the collection trap was held at room temperature (21 ± 2° C). Throughout the test period, the trap temperature for each bag remained the same, in order to avoid the confounding of variables.

Experiment 10: Volatile production by potatoes infected with ring-rot. This experiment was designed to test the volatile production characteristics of a ring-rot (Corynebacterium sepedonicum) (Spiekermann and Kotthoff) infection.

An isolate of C. sepedonicum[7] which had been maintained on nutrient-broth/yeast extract agar slants at 2° C was used as the test pathogen. Inoculum was prepared by inoculating sterile 50 ml swirl cultures of standard nutrient broth[8]. After 48 h of incubation at 21 ± 2° C, the cultures reached a population of approximately $3.0 \times 10^5$ colony forming bodies/ml. "Diseased" treatments were prepared by inoculating 'Russet Burbank' tubers in the previously described manner. Three replicates of the "diseased" (RR) and

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[7]Provided by G.A. Nelson, Canada Department of Agriculture Research Station, Lethbridge, Alberta, Canada.

[8]Laboratory Guide for the Identification of Plant Pathogenic Bacteria. ed. N.W. Schaad.

"wounded control" (CO) treatments were prepared.

The procedure used for the collection and analysis of the C. sepedonicum volatile output were the same as those used in the soft-rot experiments.

Experiment 11: Analysis of volatile profiles of bacterial cultures. Standard potato dextrose[9] swirl cultures were inoculated with E. carotovora bacteria in the previously described manner. The swirl cultures were sealed with foam rubber stoppers. The cultures were maintained at 22° C. At 24 h intervals, a syringe was used to collect a 10 $cm^3$ sample of the swirl culture headspace. As the needle from the syringe could be inserted through the foam stopper, the risk of contaminating the culture during the volatile sampling was minimal. The headspace samples were introduced directly into the gas chromatograph.

In one trial, E. carotovora var. atroseptica and E. carotovora var. carotovora cultures were tested. Three replicates were prepared for each treatment. In another trial, a mixed E. carotovora culture was prepared by inoculating each swirl culture with isolates of both E. carotovora varieties. Three replicates of this treatment were tested against three non-inoculated treatments.

Bag Sampling Procedure

Direct sampling of the bag atmospheres could not provide adequate data due to the low concentration of metabolic volatiles in the bag headspace. A trapping/concentration step designed to yield a concentrated but representative sample of the bags' headspace atmosphere was necessary. After testing a number of trapping systems, a modified version of the inlet liner/trap system developed by Brown et al. (1972) was selected as the optimal

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[9]Laboratory Guide for the Identification of Plant Pathogenic Bacteria. ed. N.W. Schaad.

trapping system for our particular experimental situation. This system utilizes a gas chromatography injector port liner packed with one of the porous polymer absorbents as a one step trapping/concentration apparatus.

In our experiment, traps were prepared from sections of acid etched pyrex glass tubing (4.0 cm long x 3 mm O.D., 1.75 mm I.D.), flanged at one end in a manner similar to the standard Hewlett Packard injector liners. The flange ensures an efficient gas-tight seal between the top of the trap and the lip of the injector port.

The porous polymer absorbent Chromosorb 105[10] was used in the traps. When packed with 180 mg of 60/80 mesh Chromosorb 105, the traps had an effective trapping surface of 7.8 to 9.1 $m^2$. The traps were vibrated during filling to promote uniform packing of the absorbent. The polymer beads were held in place within the trap by plugs of silanized glass wool. Prior to their first use, the traps were conditioned by inserting them into the heated injector port of the gas chromatograph (220° C) and allowing the carrier gas stream (high purity $N_2$ at 30 ml/min) to flow through the trap for 24 h.

All bag headspace samples were collected by introducing the trap into a line (5 mm O.D. Tygon and Teflon tubing) connecting the sample bag with a vacuum pump (Figure A). Sampling involved passing a known volume of the headspace air through the trap at a controlled rate. As the air sample passed through the trap, the volatiles entrained in the headspace sample were absorbed onto the porous polymer beads. In all bag experiments, the bags were sampled for 15 min. at a flow rate of 120 ml/min, resulting in a final sample size of 1.8 L/trap. An in-line needle valve[11] was used to regulate the sampling rate. Flow rates were determined by water displacement.

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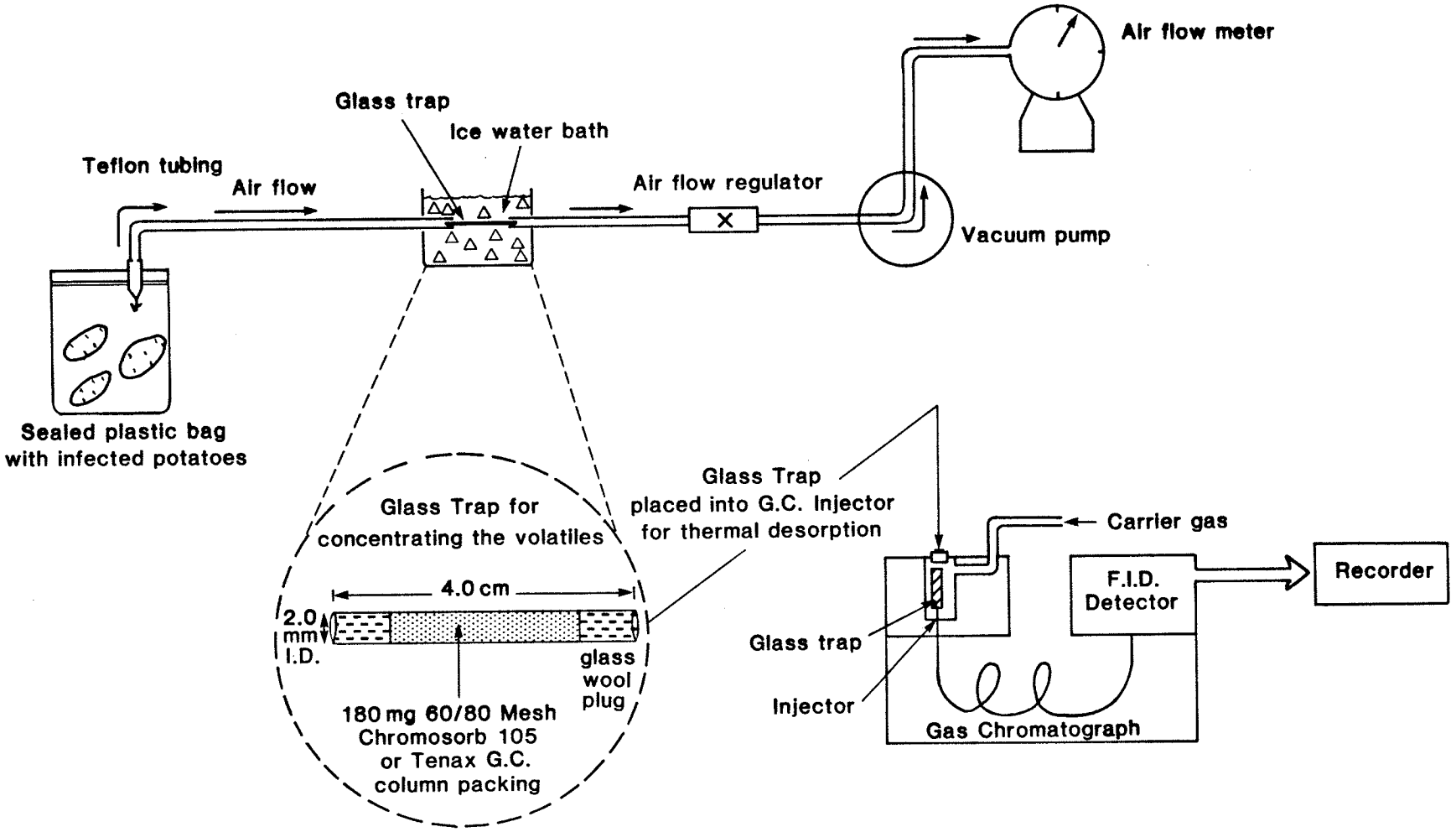
[10]Johns-Manville Co., Port Credit, Ontario, Canada.

[11]Co. No. IVM4, Whitey Co., Oakland, California, U.S.A.

FIGURE A. Technique for trapping and identification of volatiles produced by potatoes infected with disease organisms.



Unless otherwise specified, the traps were maintained in an ice-water bath (1° C) during the sampling period.

Sample Introduction

Generally, the traps were transferred to the gas chromatograph immediately after the sampling procedure was completed. However, when properly capped and refrigerated (-2.5° C), the traps could be stored for several days without appreciable volatile loss.

The traps were introduced into the gas chromatograph by removing the septum retainer nut and inserting the trap into the pre-heated injector (200° C) until the flanged neck of the trap came into contact with the countersunk top of the injector column. The septum nut was immediately replaced and the chromatographic analysis (temperature program, peak integration) was commenced. When the trap is introduced into the heated injector port, the compounds entrained on the polymer beads are instantaneously thermally volatilized. The normal carrier gas stream, which runs through the injector, sweeps the desorbed volatiles from the trap onto the analytical column.

The traps were retained in the injector throughout the 20 to 30 min gas chromatograph run. During this period the continuous flushing action of the heated carrier gas purged any low volatility compounds that might have remained on the trap. The regenerated traps were allowed to cool for 24 h prior to the next trapping/desorption cycle.

Chromatographic Conditions

All analyses were performed using a Hewlett Packard 5720A single column gas chromatograph, equipped with a flame ionization detector. This unit was designed to accept a glass injector port liner.

A stainless steel column (3.3 m long, 5 mm O.D., 3 mm I.D.) packed with

3.6 g of 50/80 mesh Porapak P[12] was used in all chromatographic analyses. The column was packed under suction and vibration, then capped with silanized glass wool plugs. The column was conditioned with a $N_2$ purge flow for 48 h at 220° C prior to use.

Injector port and detector temperatures were 200° and 220° C, respectively. The flow rate of the high purity $N_2$ carrier gas was 30 ml/min. In order to obtain adequate peak resolution with reasonable retention times, an oven temperature program was used in all G.C. analyses ($T_{zero}$ 100° - $T_{final}$ 200° C; at 4°C/min in jar experiments, 6° C/min in bag experiments).

The gas chromatograph was coupled to a Columbia Scientific Industries 204 automatic integrator which determined the retention times and peak areas of the individual peaks. This unit also calculated the total peak area/chromatogram, and also listed the normalized abundance of the individual peaks (area of individual peak/total peak area of chromatogram x 100). A Perkin Elmer 105 strip chart recorder was used to trace the chromatogram and a Victor Digitmatic event recorder was used to produce a hard copy of the integrator information.

Compound Identification

Tentative identification of the chromatogram peaks was based on the comparison of the peak retention times with the retention times of known standards. Volatile samples of the standards were prepared by evaporation of 1 µl of the compound in a 2 ml septum-sealed reaction vial[13]. A 0.25 $cm^3$ sample of the atmosphere within the vial was removed with a gas-tight syringe and injected directly into the gas chromatograph. At each oven program rate

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[12]Waters Associates, Milford, Mass., U.S.A.

[13]Suppelco Inc., Bellafonte, PA, U.S.A.

(4 and 6° C/min), the retention time of each standard was determined in three separate tests. A wide range of $C_1$-$C_5$ aliphatics, aldehydes, ketones, alcohols, esters, and organic acids were tested[14]. In some cases, two or more compounds had column retention times that matched the retention time of one experimental peak. As it was not possible to ascertain whether the experimental peak represented more than one compound all the potential matching compounds were included in the lists of the tentative compound identities.

The retention indices obtained from these tests were correlated with previously published retention data for volatile organics on Porapak P (Dave 1969; Supina and Ross 1969; Anon 1977) as an additional check on the compound identities.

In order to determine whether the introduction of volatiles by desorption from the polymers traps had an impact on retention times, a 1 µl aliquot of the standard was absorbed onto polymer traps prior to the trap being introduced into the gas chromatograph. The retention times obtained were compared with the values obtained for direct sample injection.

Bacteria Recoveries

E. carotovora recovery. At the end of each test, a 0.1 ml aliquot of the bacterial seepage was removed from each incubation vessel. This material was diluted ($1 \times 10^{-4}$) with sterile distilled water. A 0.1 ml aliquot of this solution was plated on crystal violet pectate (CVP)[15] medium. The plates were incubated for 72 h at 27° C. E. carotovora bacteria were identified by the formation of translucent, irridescent, deeply-pitted colonies.

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[14]Chem-Services Inc., West Chester, Pa.

[15-18]Laboratory Guide for the Identification of Plant Pathogenic Bacteria. ed. N.W. Schaad.

Duplicate tests were conducted for each experimental replicate.

E. carotovora variety isolation. Ten independent E. carotovora colonies were selected at random from each of the CVP plates prepared for the disease replicates at the termination of each experiment. These colonies were spotted on methyl-D-glucoside/agar plates[16]. The plates were incubated at 20° C for 48 h. The presence of a red tinge to the colonies was considered a positive reaction, diagnostic for E. carotovora var. atroseptica.

Secondary microflora identification. At the end of each test, another 0.1 ml aliquot of bacterial seepage was removed from the incubation vessel. This material was diluted ($1 \times 10^{-4}$) with sterile distilled water. A 0.1 ml aliquot of this solution was plated on nutrient agar medium[17]. The plates were incubated for 48 h at 27° C. Microflora identifications were based entirely upon colony morphology, and were consequently only tentative identifications.

C. sepedonicum recovery. At the end of the test, a 0.1 ml aliquot of bacterial seepage was collected from each incubation vessel. This material was diluted ($1 \times 10^{-4}$) with sterile distilled water. A 0.1 ml aliquot of this solution was plated onto CVS medium[18], which is selective for coryne-bacteria. The plates were incubated for 72 h at 22° C. C. sepedonicum bacteria were identified as colorless colonies on the selective medium.

Statistical Tests and Data Manipulation

Major chromatogram peaks were defined as any compound which occurred at an average level of > 1000 integrator units in any of the experimental treatments.

The mean values for the peak area and percentage of total peak area (PTPA) values for the major chromatogram peaks were calculated as follows:

$$\bar{X}\ \text{PEAK AREA}\ (\text{PEAK}_i) = \sum_{\text{DAY 1}}^{\text{DAY X}} \frac{\text{DAILY PEAK AREA FOR PEAK}_i}{\text{NUMBER OF DAYS IN INCUBATION PERIOD}}$$

$$\bar{X}\ \text{PTPA}\ (\text{PEAK}_i) = \frac{\left[\sum_{\text{DAY 1}}^{\text{DAY X}} \frac{\text{DAILY PEAK AREA FOR PEAK}_i}{\text{TOTAL DAILY PEAK AREA}}\right] \text{X } 100}{\text{NUMBER OF DAYS IN INCUBATION PERIOD}}$$

This method of calculating the PTPA values was intended to de-emphasize peaks that appeared at elevated levels for short duration. Spurious peaks that became prominant late in the infection were a particular concern. The exponential increase in volatile production over the incubation period meant that late developing compounds could overshadow components that had played a stable role throughout the majority of the incubation period and which were, therefore, considered better disease indicators. Additionally, in the final stages of the infection, it is possible that the volatile output could be altered by changes in the availability of substrate suitable for the pathogen and also by the development of secondary saprophytic micro-organisms. These factors would tend to reduce the validity of late developing compounds as accurate indicators of a specific disease.

With this system of PTPA calculation the summation of the PTPA values

for all the peaks in any treatment will not necessarily equal 100%. In treatments where transient peaks or compounds averaging output levels of less than 1000 integrator units formed a significant percentage of the total volatile output, the summation of the PTPA values may be relatively small. Further with this system of PTPA calculation the results for Duncan's tests on the PTPA values will not necessarily be identical to the results for tests of the peak area values.

The analysis of variance and Duncan's multiple range tests were performed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) programs. In cases where block sizes were uneven the Duncan's test procedures were based on a calculated harmonic mean for cell size.

To provide a means of using volatiles as identifiers for a particular disease, treatment signatures or fingerprints were prepared according to the system developed by Henis et al. (1966). The letters designating the peaks were arranged in an order corresponding to decreasing peak areas or decreasing PTPA values. Metabolites occurring in trace amounts (average area $<$ 200 integrator units) were designated by lower case letters and were arranged in alphabetical order.

The statistical significance of the differences between the peaks making up the signatures was determined by the use of Duncan's multiple range test at the 5% confidence level. Peaks which were not significantly different were enclosed by a set of brackets. The differences between those not so enclosed are significant at the 5% level. The trace level peaks were not considered in the statistical analysis.

Based on Henis' et al. (1966) system, the treatment signatures could be distinguished if any of the following applied: 1) two treatments had the peak in common but the peak area or PTPA for that peak were statistically different; 2) one of the treatments produced the metabolite either in signi-

ficant or trace amounts, whereas the compound was not found in any replicates of the second treatment; 3) one of the treatments produced the compound in significant quantities whereas the other produces only a trace, however, if the peak was in the lowest homologous group in the Duncan's test a difference with a trace peak was not considered significant.

In the analysis of the volatile data a number of data variables were established, i.e., total volatile production, total number of peaks, etc. The use of the word 'total' in these variables is not meant to imply that the trapping and detection system was capable of providing information on all the volatile compounds being produced by the diseased potatoes. These variables only refer to the volatile compounds that could be contained by the trapping/sampling systems and detected using a flame ionization detector.

# RESULTS

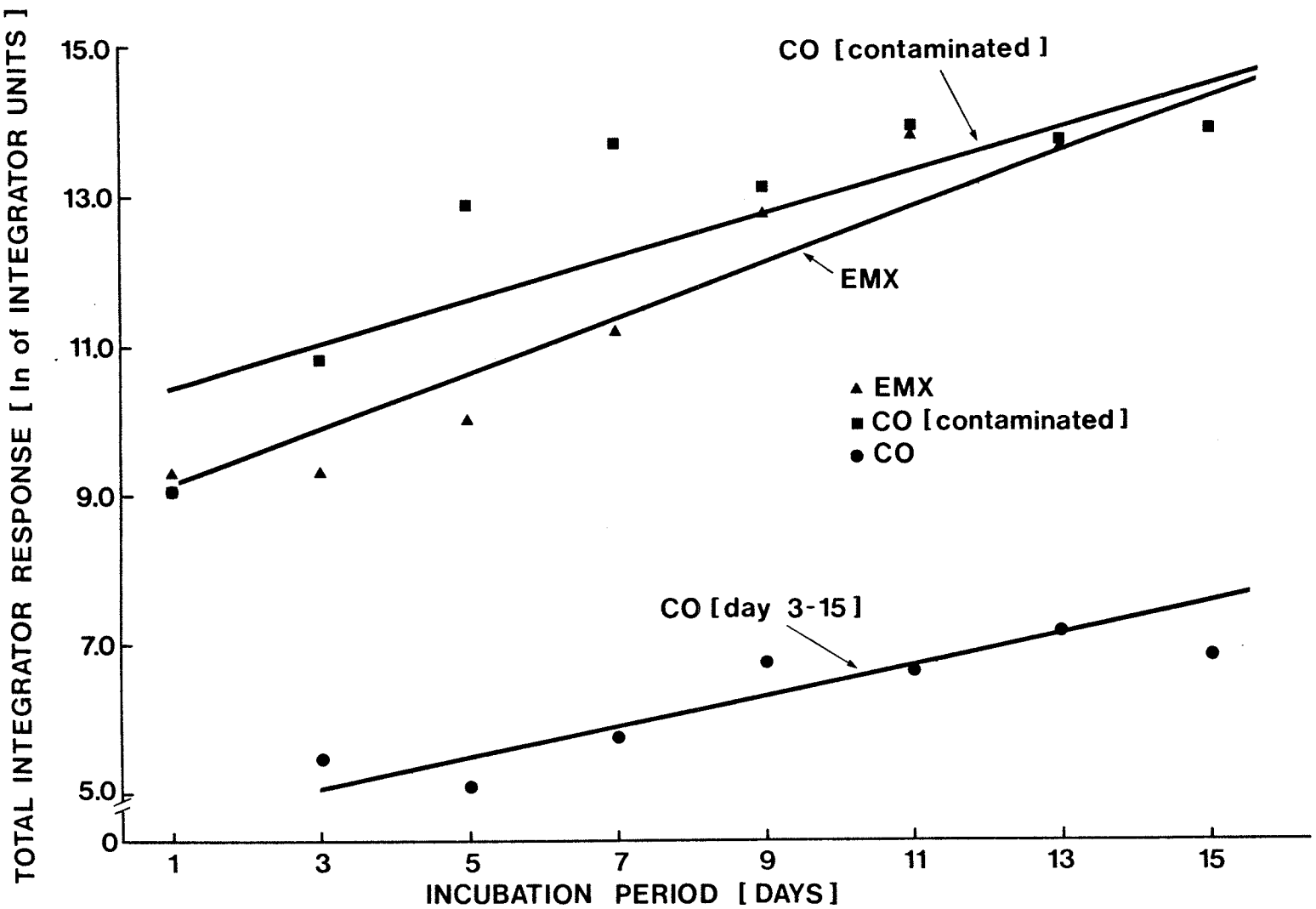
## Experiment 1: Volatile Profiles of a Mixed *E. carotovora* Infection

Disease summary. The *E. carotovora* infected treatments (EMX) appeared to be relatively uniform in terms of their visual symptomology. The first obvious signs of decay appeared on day 8 of the incubation period, with pitting of the tuber surfaces and the beginning of seepage from the inoculation points. These symptoms progressed and by day 15 the potatoes had lost all structural integrity. On day 7 one of the wounded controls (CO) began to show disease symptoms very similar to those observed in the infected treatments. This infection eventually resulted in the total decay of the tuber. At the termination of the experiment, both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* bacteria were isolated from this treatment. The remaining control treatments remained healthy throughout the incubation period.

At the end of the incubation period, *E. carotovora* bacteria were isolated from all inoculated treatments. Tests indicated that both varieties of *E. carotovora* were present in all the diseased treatments. Populations of secondary organisms were minimal in comparison with the *E. carotovora* population. Yeast, *E. coli*, and *Clostridium* were identified as the predominant secondary microflora.

Volatile production. The mean daily volatile production data for a mixed *E. carotovora* infection are presented as an exponential function in Figure 1a. The apparently contaminated wounded control was plotted separately.

FIGURE 1a. Daily total volatile production and corresponding best-fit lines for a mixed E. carotovora (EMX) infection.


TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
15.0
13.0
11.0
9.0
7.0
5.0
0
1
3
5
7
9
11
13
15
INCUBATION PERIOD [ DAYS ]
CO [contaminated ]
EMX
CO [day 3-15 ]
▲ EMX
■ CO [contaminated ]
● CO

The total volatile output for the EMX treatment increased steadily until day 11 of the incubation period, at which time production levelled out. Total volatile production at the end of the experiment was 160X greater than at the onset.

The mean daily volatile output of the contaminated control followed a production pattern very similar to that recorded for the EMX treatment. Volatile production levels at the end of the experiment and the degree of increase in volatile production over the experimental period (163X) were similar to the results for the EMX treatment.

The daily volatile output of the non-contaminated controls followed a bimodal response curve. Volatile output peaked at day 1, declined through to day 5, then increased steadily through the remainder of the incubation period. The volatile output at the end of the incubation period was only 10% of the volatile output on day 1.

The linear regression equations for the natural logs of the daily volatile production values of the EMX and CO treatments are presented in Table 1a. The regression line for the healthy controls was based on the data from days 3 to 15. The significance of the regression coefficients indicated that the total daily volatile production of the EMX ($P = 0.01$) contaminated control ($P = 0.05$) and healthy control ($P = 0.05$) treatments showed an exponential pattern of increase over the incubation period.

Table 1b contains the test statistics for the covariant analyses of the daily volatile production regression lines. The regression equations for the EMX treatments and the healthy controls had significantly different slopes ($P = 0.05$) and general equations ($P = 0.01$). The healthy and the contaminated controls could also be differentiated on the basis of both the regression slopes ($P = 0.01$) and general equations ($P = 0.01$). The regression equations

TABLE 1a. Linear regression equations for the daily total integrator responses of a mixed E. carotovora infection.

| Treatment | Equation | $r^2$ |
|---|---|---|
| E. carotovora infected | $Y^{1)} = e^{3.66+0.38\bar{X}^{2)}}$ | $.93^{**}$ |
| "Contaminated" control | $Y = e^{10.23+0.29\bar{X}}$ | $.71^{*}$ |
| "Healthy"[3] control | $Y = e^{4.39+0.21\bar{X}}$ | $.95^{**}$ |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

3) Day 1 not included due to "wound" effect.

** Significant at the 1% level.

* Significant at the 5% level.

TABLE 1b. Analysis of covariance for the linear regression equations calculated for the total integrator responses[1] of a mixed *E. carotovora* infection.

| Pairwise comparison | F Test for slope | F Test for similarity between lines |
|---|---|---|
| *E. carotovora* vs Contaminated control | $0.89^{N.S.}$ | $4.41^{N.S.}$ |
| Contaminated control vs Healthy control | $13.43^{**}$ | $68.66^{**}$ |
| *E. carotovora* vs Healthy control | $7.07^{*}$ | $342.55^{**}$ |

$^{**}$Differences significant at the 1% level.

1) Ln of integrator units.

for the EMX treatments could not be differentiated from the contaminated control on the basis of either the regression equation slopes or general formulations ($P = 0.05$).

The mean of the peak area totals ($LOG_{10}$) for the EMX treatments was approximately 130X greater than that for the controls (Table 1c). This difference was significant at the 1% level.

The major peaks in the volatile profiles of the experimental treatments are listed in REFERENCE TABLE A. The mean number of peaks/chromatogram for the EMX treatments was significantly ($P = 0.01$) greater than for the controls, even with the contaminated control data included in the analysis (Table 1d).

The areas ($LOG_{10}$) and PTPA values, averaged over the entire incubation period, of the major peaks in the treatment chromatograms are presented in Table 1e. The chromatogram signatures for the peak areas and PTPA values are presented in Table 1f (REFERENCE TABLE B for peak identification). The metabolites which, according to the test statistics in Table 1e could potentially be used to differentiate between the treatments, are listed in Table 1g. Based on average compound production levels, compound F, 1-butanal/2-butanol, 2-butanone/2-methyl-1-propanol, and compound K could be used to differentiate between the volatile profiles of the healthy controls and both the EMX and the contaminated control treatments. In all cases, the healthy controls produced significantly lower quantities of the diagnostic metabolites. The same group of compounds could also be used to differentiate between the healthy controls and both the EMX and the contaminated control treatments when the PTPA values were examined. Again all differences involved greater relative volatile outputs by the EMX and contaminated control treatments. The EMX treatment could not be differentiated from the contaminated control on the basis of either the area of PTPA values of any of the metabolites recorded in this experiment.

TABLE 1c. Pairwise comparison of means of peak area totals[1)] averaged over the incubation period for a mixed E. carotovora (EMX) infection.

| Pairwise comparison | Mean total integrator response | t Value |
|---|---|---|
| EMX | 5.947 | 4.232** |
| vs | | |
| Control | 3.838 | |

**Values significantly different at the 1% level.

1) Log of sum of all individual peak areas.

TABLE 1d. Pairwise comparison of mean numbers of peaks/chromatogram over the incubation period for a mixed E. carotovora (EMX) infection.

| Pairwise comparison | Mean number peaks/chromatogram | t Value |
|---|---|---|
| EMX | 7.2 | 9.04** |
| vs | | |
| Non-Inoculated | 2.4 | |

**Values significantly different at the 1% level.

REFERENCE TABLE A. Volatile peaks occurring in the jar experiments (Experiments 1-3).

| Retention time (min.) | Compound identity | Volatile Detectable In: | | | | | |
|---|---|---|---|---|---|---|---|
| | | *E. carotovora* var. *atroseptica* | *E. carotovora* var. *carotovora* | EMX | Control | Russet Burbank | Norland |
| 2.7 | Methanol | * | * | * | * | * | * |
| 3.7 | Ethanal | * | * | * | | * | * |
| 5.4 | Ethanol | * | * | * | * | * | * |
| 6.7 | 2-Propanol | * | * | * | | * | * |
| 7.4 | 1-Propanal/ 2-Propanone | * | * | * | * | * | * |
| 8.2 | ? | * | * | * | | * | * |
| 9.7 | 1-Propanol | * | * | * | * | * | * |
| 12.1 | 1-Butanal/ 2-Butanol | * | * | * | * | * | * |
| 13.0 | 2-Butanone 2-Methyl-1-Propanol | * | * | * | * | * | * |
| 14.7 | 1-Butanol | * | * | * | * | * | * |
| 16.0 | ? | * | * | * | | * | * |
| 16.6 | 3-Hydroxy-2-Butanone | * | * | * | * | * | * |
| 18.5 | ? | * | * | * | | * | * |

* = Detected in treatment.

TABLE 1e. Areas of chromatogram peaks averaged over the incubation period for a mixed E. carotovora (EMX) infection.

| | Ethanal | | Ethanol | | 1-Propanal/ 2-Butanone | | RT = 8.2 min | | 1-Propanol | | 1-Butanal/ 2-Butanol | | 1-Butanol | | 3-Hydroxy- 2-Butanone | | RT = 18.5 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| EMX | 1.26 a* | 1.05 a | 3.48 a | 9.56 a | 4.03 a | 11.45 a | 2.30 a | 2.30 a | 3.01 a | 2.07 a | 2.76 a | 1.45 a | 3.69 a | 7.58 a | 1.51 a | 1.42 a | 2.11 a | 0.86 a |
| Contaminated control | 4.18 a | 2.21 a | 5.26 a | 16.07 a | 5.30 a | 2.62 a | 3.95 a | 0.57 a | 4.67 a | 2.86 a | 4.41 a | 1.69 a | 5.19 a | 9.60 a | 4.20 a | 1.26 a | 2.12 a | 0.41 a |
| Healthy control | 2.39 a | 1.05 a | 3.03 a | 5.14 a | 3.13 a | 7.43 a | T b | T b | 2.40 a | 0.48 a | T b | T b | 3.54 a | 15.00 a | T b | T b | 2.53 a | 0.71 a |

[1] Total area (Log of integrator units).

[2] Relative area = individual peak area/total peak area X 100.

* Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test for ordered means.

T = Trace.

TABLE 1f. Chromatogram signatures of a mixed E. carotovora (EMX) infection and for contaminated and non-contaminated controls. (After the system of Henis et al. 1966 p. 33)

| Treatment | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| EMX | E J C G H F I M B L A K D | E J C G H F I M B L A K D |
| Contaminated control | E C J G H I L B F M K A : d | E C J G H I L B F M K : ad |
| Non-contaminated control | J E C G M B L K H A : dfi | J E C G M B L K H A : df |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 1g. Comparison between chromatogram signatures for a mixed E. carotovora (EMX) infection and for contaminated and non-contaminated controls.

| | Characters Distinguishing Between Treatments[a] | | | |
|---|---|---|---|---|
| | EMX | | Contaminated Control | |
| Treatment | Area[b] | PTPA[c] | Area | PTPA |
| EMX | . | . | * | * |
| Non-contaminated control | FHIK | FHIK | FHIK | FHIK |

a) Characters which differ significantly between treatments.

b) Log peak area.

c) Percentage total peak area.

* No factors significantly different.

REFERENCE TABLE B. Tentative identification of chromatogram peaks listed in chromatogram signatures.

Jar Experiments (EXPT 1-3)

A = Methanol

B = Ethanal

C = Ethanol

D = 2-Propanol

E = 1-Propanal/2-Propanone

F = Unidentified (RT = 8.2 min)

G = 1-Propanol

H = 1-Butanal/2-Butanol

I = 2-Butanone/2-Methyl-1-Propanol

J = 1-Butanol

K = Unidentified (RT = 16.0 min)

L = 3-Hydroxy-2-Butanone

M = Unidentified (RT = 18.5 min)

## Experiment 2: Volatile Profiles of Potato Cultivars and E. carotovora Varieties

Disease summary. Disease development amongst the treatment replicates was relatively uniform. There were no symptomological differences between the E. carotovora varieties tested. Typical signs of E. carotovora infection were apparent by day 5 of the incubation period, with pitting of the tuber surfaces and the appearance of seepage from the inoculation points. By day 15, the tuber tissues had been completely disrupted by the disease. The control treatments remained in good condition throughout the test.

E. carotovora bacteria were present in the bacterial seepage of all infected samples at the end of the incubation period. Tests indicated that none of the variety specific infections had become contaminated by the opposing variety of E. carotovora. Plating of the bacterial seepage showed that populations of secondary microflora were relatively small in comparison to the E. carotovora populations. Staphylococcus and various yeasts dominated the secondary microflora.

Volatile production. In all statistical tests in this experiment, the disease treatment X potato cultivar interactions were non-significant. Consequently, only the main effect variables were analyzed in detail.

Schematic chromatograms representing the peak areas and percentages of total peak areas (PTPA) (Figure 2a) illustrate a number of the changes that occur in the potatoes' volatile profile during the progression of an E. carotovora infection. Volatile output, as measured by both the number of peaks in each day's chromatogram and the total amounts of the various components produced, increased steadily during the progression of the disease. In the diseased treatments (ECC and ECA), the number of peaks/chromatogram increased steadily from an average of 3 peaks at day 1 to approximately

FIGURE 2a. Areas and PTPA* values for chromatogram peaks at three stages in incubation period of E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

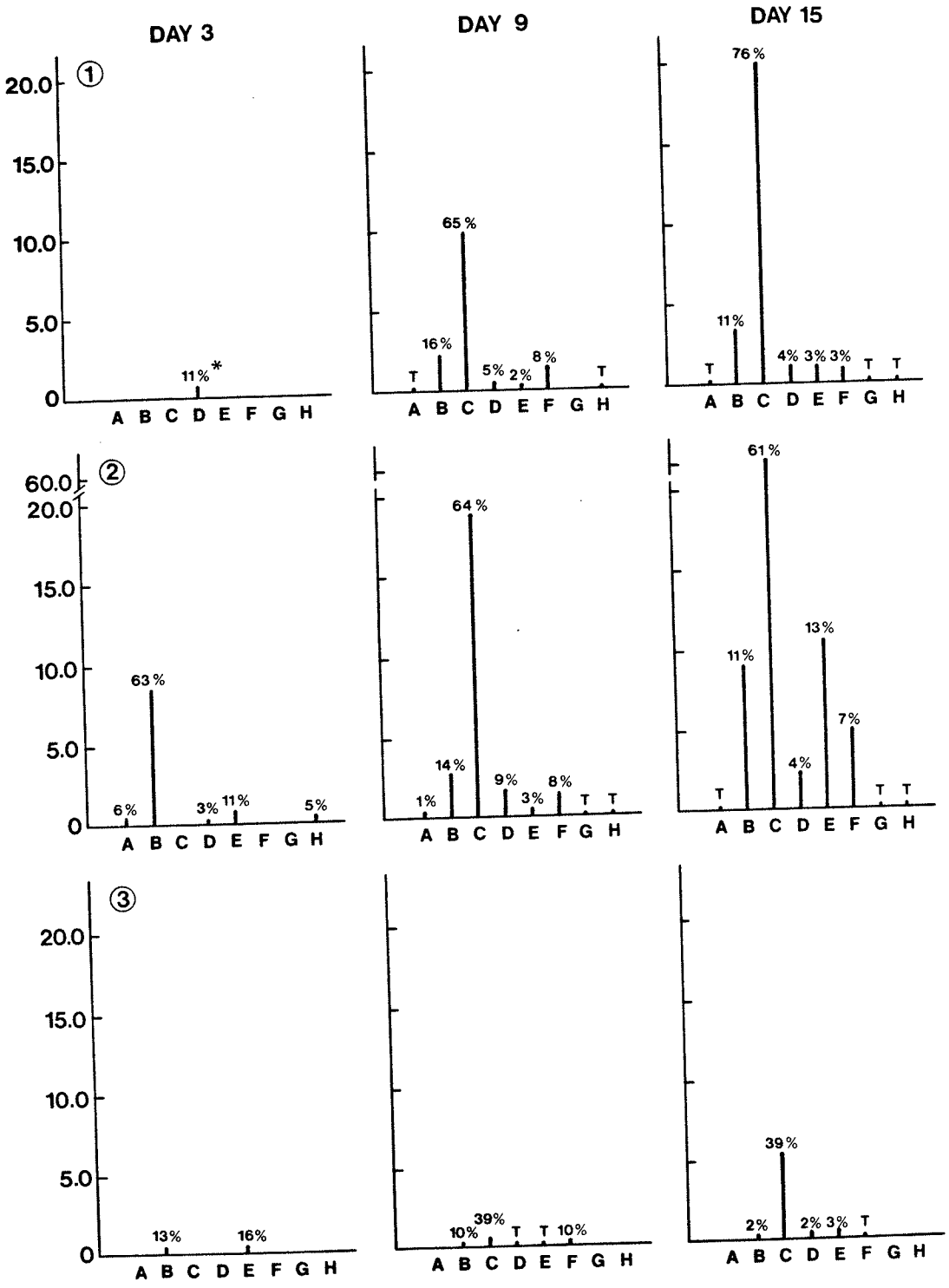
1) E. carotovora var. atroseptica
2) E. carotovora var. carotovora
3) Control

*Percentage of total peak areas.

Peak A = Ethanal
B = Ethanol
C = 1-Propanal/2-Propanone
D = 1-Propanol
E = Butyraldehyde/Sec-Butanol
F = 1-Butanol
G = 3-Hydroxy-2-Butanone
H = ?

X Axis = Peaks

Y Axis = Integrator Units X $10^{-4}$


DAY 3
DAY 9
DAY 15
①
20.0
15.0
10.0
5.0
0
11%*
16%
65%
5%
2%
8%
T
11%
76%
4%
3%
3%
T
A B C D E F G H
②
60.0
20.0
15.0
10.0
5.0
0
6%
63%
3%
11%
5%
1%
14%
64%
9%
3%
8%
T
11%
61%
13%
7%
4%
③
13%
16%
10%
39%
2%
3%

10 peaks by day 15 (Table 2a). By day 15, the total daily volatile output for the diseased treatments had increased 36X over the levels on day 1 (Table 2b).

In many cases, the individual chromatogram peaks did not respond identically over the duration of the experiment (Figure 2a). For example, the 1-propanol/2-propanone peak (Peak C) did not appear until midway through the disease infection. From that point onwards, output kept pace with the general increase in volatile production. This is reflected by the relatively stable PTPA values. By contrast, 1-propanol (Peak D) was present throughout the infection. However, its production levels did not increase significantly over the duration of the test period. Consequently, its PTPA values generally declined over the course of the infection.

The total daily volatile outputs of the diseased and control treatments are plotted as a normal function in Figure 2b and as an exponential function in Figure 2c. The total daily volatile output data for the disease treatments showed a very good fit ($P = 0.01$) to the derived exponential regression equations (Table 2c).

The volatile profiles for the controls were clearly distinguishable from those of the diseased treatments. Although there was considerable overlap in the compounds detected in the treatments' volatile profiles, several compounds (ethanal and four unidentified compounds; RT = 6.7, 8.2, 16.0, and 18.5 min) present at significant levels in the infected treatments could not be detected in the controls (REFERENCE TABLE A). The number of peaks/chromatogram in the controls did increase somewhat over the incubation period (Table 2a). However, at each point in the incubation period, the number of peaks in the control chromatograms was lower than for the diseased treatments (Table 2a). When averaged over the entire incubation period, this difference was significant at the 1% level (Table 2d).

TABLE 2a. Number of peaks/chromatogram for 'Russet Burbank' (R) and 'Norland' (N) tubers infected with E. carotovora var. carotovora (CC) and E. carotovora var. atroseptica (CA). (Wounded control = CO)

| Treatment | Incubation Period, Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 89 |
| RCA | 2 | 1 | 6 | 3 | 9 | 9 | 10 | 11 |
| NCA | 1 | 1 | 2 | 3 | 6 | 7 | 8 | 9 |
| RCC | 4 | 6 | 8 | 9 | 10 | 9 | 10 | 9 |
| NCC | 4 | 7 | 8 | 10 | 9 | 10 | 10 | 10 |
| RCO | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 |
| NCO | 1 | 1 | 1 | 3 | 5 | 5 | 5 | 4 |
| CA | 1.5 | 1.0 | 4.0 | 3.0 | 7.5 | 8.0 | 9.0 | 10.0 |
| CC | 4.0 | 6.5 | 8.0 | 9.5 | 9.5 | 9.5 | 10.0 | 9.5 |
| $\bar{X}$ = Diseased | 2.8 | 3.8 | 6.0 | 6.3 | 8.5 | 8.8 | 9.5 | 9.8 |
| CO | 0.5 | 0.5 | 0.5 | 1.5 | 2.5 | 3.5 | 3.5 | 3.0 |
| R | 2.0 | 2.3 | 4.6 | 4.0 | 6.3 | 6.6 | 7.3 | 7.0 |
| N | 2.0 | 3.0 | 3.6 | 5.3 | 5.0 | 7.3 | 7.6 | 7.6 |

TABLE 2b. Relative total peak area[1)] for 'Russet Burbank' (R) and 'Norland' (N) tubers infected with E. carotovora var. carotovora (CC) and E. carotovora var. atroseptica (CA). Wounded control = CO.

| | Incubation Period, Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 |
| RCA | 0.5 | .1 | 1.8 | 13.4 | 11.4 | 30.6 | 35.2 | 51.3 |
| NCA | 0.2 | .3 | .8 | 5.2 | 12.9 | 19.3 | 33.2 | 47.3 |
| RCC | 2.5 | 17.2 | 15.7 | 36.6 | 39.6 | 68.3 | 90.4 | 94.0 |
| NCC | 4.8 | 11.2 | 10.6 | 44.0 | 17.9 | 77.6 | 79.4 | 100.0 |
| RCO | .1 | .1 | .1 | .1 | .1 | 1.8 | .1 | .1 |
| NCO | .1 | .1 | .1 | 0.6 | .1 | 10.1 | 8.3 | 11.3 |
| | | | | % | | | | |
| CA | 0.4 | 0.2 | 1.3 | 9.3 | 12.2 | 25.0 | 34.2 | 49.3 |
| CC | 3.6 | 14.2 | 8.2 | 40.3 | 28.8 | 73.0 | 84.9 | 97.0 |
| $\bar{X}$ Diseased | 2.0 | 7.2 | 4.3 | 24.8 | 20.5 | 49.0 | 59.6 | 73.1 |
| CO | .1 | .1 | .1 | 0.3 | .1 | 2.8 | 1.6 | 3.1 |
| R | 1.0 | 5.8 | 5.9 | 16.7 | 17.0 | 33.6 | 41.9 | 48.4 |
| N | 1.7 | 3.9 | 3.8 | 16.6 | 10.3 | 32.3 | 37.5 | 49.1 |

1)Total peak area of NCC at 15 days = 100.0.

2)Value = individual peak area/peak area of NCC at 15 days X 100.

FIGURE 2b. Daily total volatile production of 'Russet Burbank' (R) and 'Norland' (N) tubers with E. carotovora var. carotovora (CC) or E. carotovora var. atroseptica (CA), and control treatments (CO).

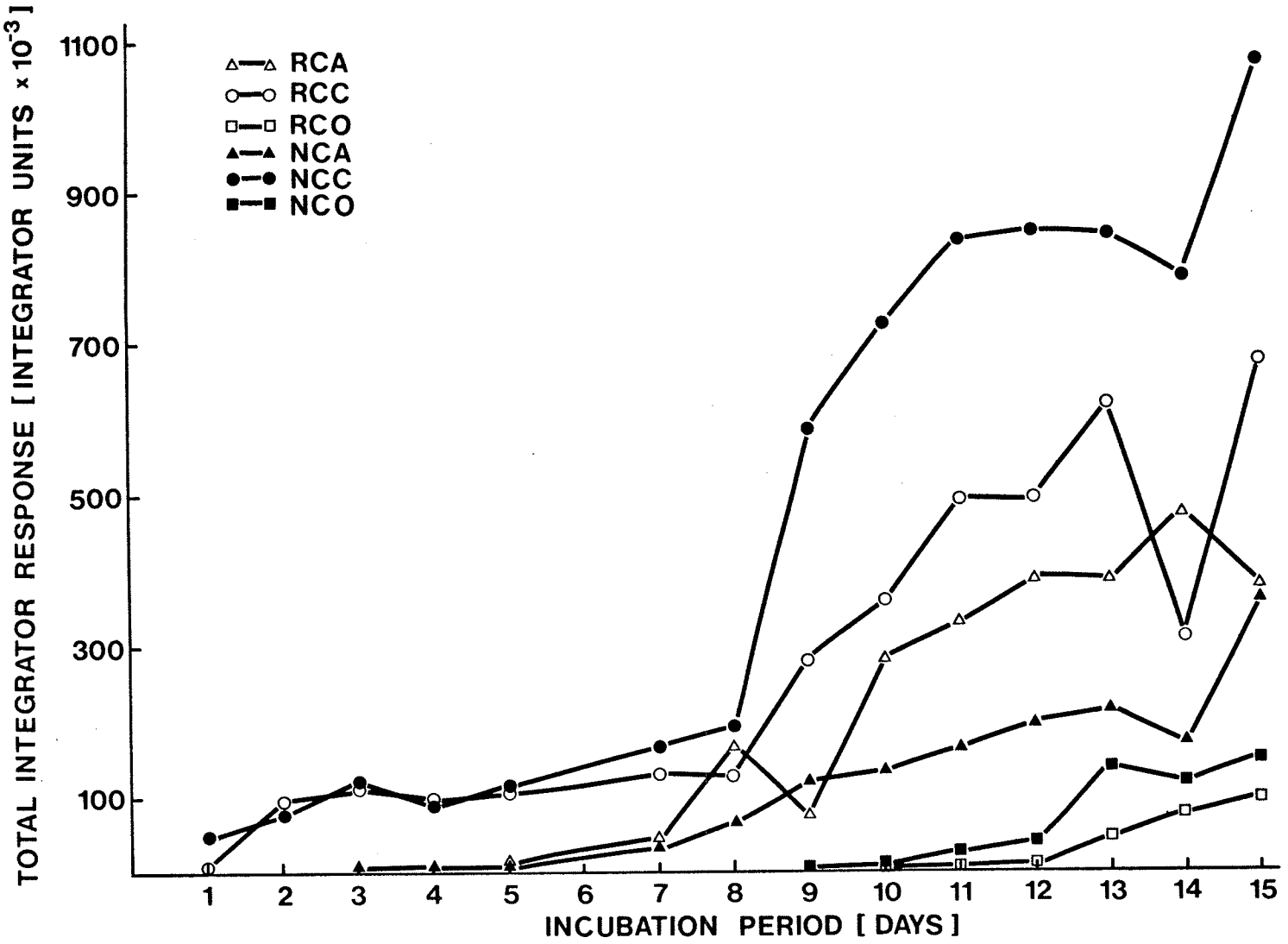

TOTAL INTEGRATOR RESPONSE [INTEGRATOR UNITS x 10$^{-3}$]
1100
900
700
500
300
100
RCA
RCC
RCO
NCA
NCC
NCO
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
INCUBATION PERIOD [ DAYS ]

FIGURE 2c. Daily total volatile production and corresponding best-fit regression equations for E. carotovora var. carotovora (ECC), E. carotovora var. atroseptica (ECA) infections, and control treatments.

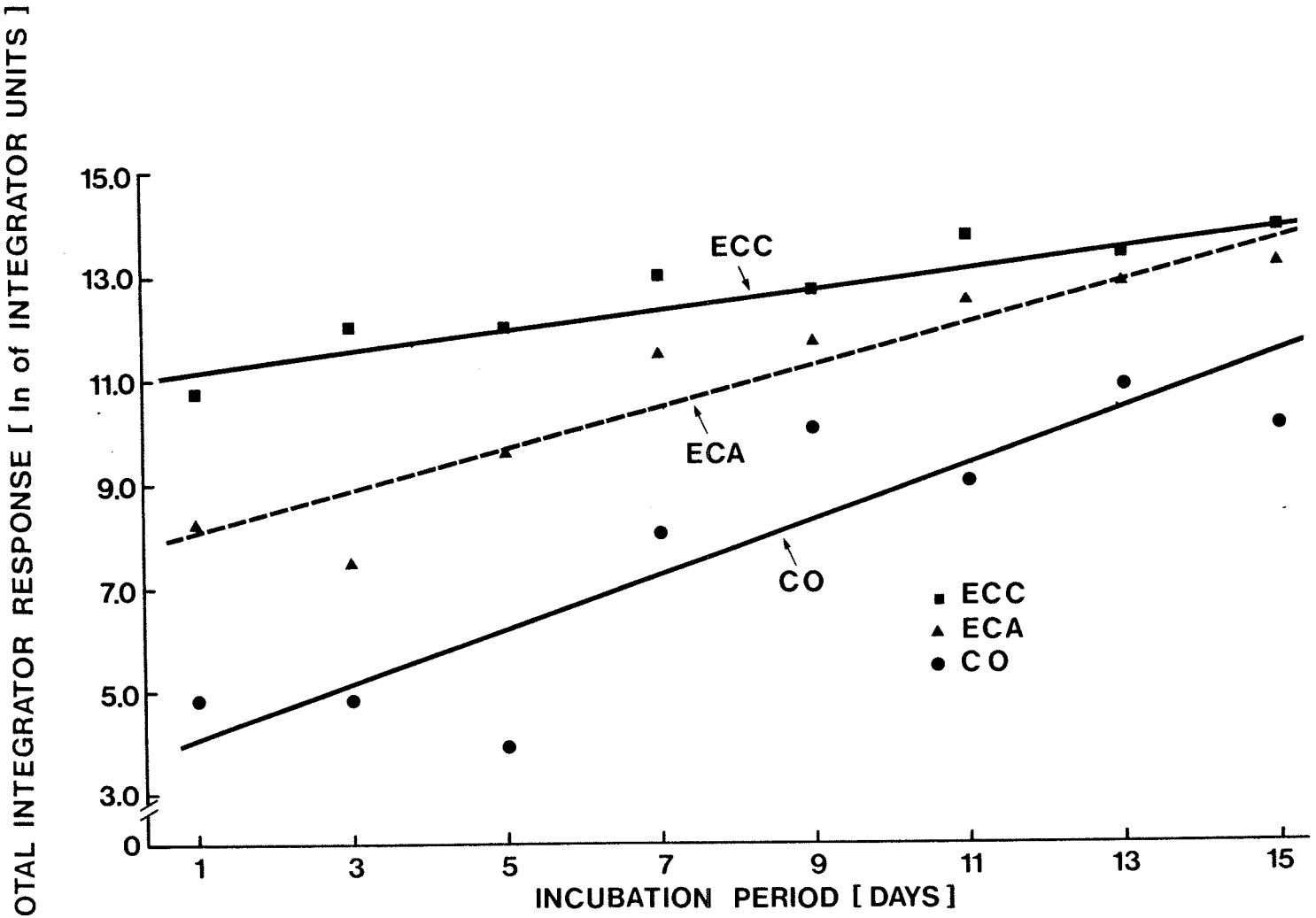

TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
15.0
13.0
11.0
9.0
7.0
5.0
3.0
0
1
3
5
7
9
11
13
15
INCUBATION PERIOD [ DAYS ]
ECC
ECA
CO
ECC
ECA
CO

TABLE 2c. Linear regression equations for the total integrator responses of E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

| Treatment | Equation | $r^2$ |
|---|---|---|
| E. carotovora var. carotovora | $Y^{1)} = e^{11.13+0.19\bar{X}^{2)}}$ | $.89^{**}$ |
| E. carotovora var. atroseptica | $Y = e^{7.62+0.41\bar{X}}$ | $.88^{**}$ |
| Control | $Y = e^{3.67+0.51\bar{X}}$ | $.79^{*}$ |

1) Y = Total integrator response.

2) $\bar{X}$ = Incubation period (days).

** Significant at the 1% level.

* Significant at the 5% level.

TABLE 2d. Pairwise comparison of mean number of peaks/chromatogram over the incubation period for E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

| Pairwise comparison | Mean number peaks/chromatogram | t Value |
|---|---|---|
| E. carotovora var. carotovora | 8.3 | |
| vs | | 3.97** |
| E. carotovora var. atroseptica | 5.5 | |
| E. carotovora var. carotovora | 8.3 | |
| vs | | 12.64** |
| Control | 1.9 | |
| E. carotovora var. atroseptica | 5.5 | |
| vs | | 6.41** |
| Control | 1.9 | |

**Values significantly different at the 1% level.

The total daily volatile output of the controls increased by a factor of 12X over the incubation period (Figure 2a; Table 2b). Most of this increase occurred within the final 5 days of the test, unlike the steady increase observed in the diseased treatments (Figure 2b). At every point during the incubation period, the total volatile output of the diseased treatments was greater than in the controls (Table 2b). Averaged over the entire incubation period, the E. carotovora infected treatment's volatile output was approximately 180X greater than the controls; a difference significant at the 1% level (Table 2e).

The regression coefficient for the controls showed that the daily volatile output followed an exponential production pattern (P = 0.05) (Table 2c). Analysis of covariance showed that both the regression equation and its slope for the controls were significantly (P = 0.01) different from the equations for the diseased treatments (Table 2f).

In the control treatments the production characteristics of individual peaks or metabolites over the incubation period showed patterns similar to those observed in the diseased treatments (Figure 2a). For some peaks (i.e. ethanol = Peak B) the daily production levels remained relatively stable throughout the incubation period. Alternately, 1-propanal/2-propanone (Peak C) increased at a rate proportional to the total volatile output, yielding relatively consistent PTPA values.

The mean peak areas ($LOG_{10}$) and PTPA values for the E. carotovora infected and control treatments are presented with the corresponding chromatogram signatures in Tables 2g and h (see TABLE B for peak identification). Table 2i summarizes the metabolites which, according to the test statistics in Table 2g, could be used to differentiate between the E. carotovora infected treatments and the controls. Based on average production levels, ethanal,

TABLE 2e. Pairwise comparisons of means of total peak areas[1] averaged over the incubation period for *E. carotovora* var. *carotovora*, *E. carotovora* var. *atroseptica*, and control treatments.

| Pairwise comparison | Mean total integrator responses | t Value |
|---|---|---|
| *E. carotovora* var. *carotovora* | 5.695 | |
| vs | | 4.798** |
| *E. carotovora* var. *atroseptica* | 5.349 | |
| *E. carotovora* var. *carotovora* | 5.695 | |
| vs | | 29.974** |
| Control | 3.417 | |
| *E. carotovora* var. *atroseptica* | 5.349 | |
| vs | | 25.422** |
| Control | | |

**Values significantly different at the 1% level.

[1] Log of sum of all individual peak areas.

TABLE 2f. Analysis of covariance for the linear regression equations calculated for the total integrator responses[1)] of the E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

| Pairwise comparison | F Test for slopes | F Test for similarity between lines |
|---|---|---|
| E. carotovora var. carotovora vs Control | 17.12** | 781.39** |
| E. carotovora var. atroseptica vs Control | 17.62** | 62.72** |
| E. carotovora var. carotovora vs E. carotovora var. atroseptica | 10.73** | 19.35** |

**Differences significant at the 1% level.

1) Ln of integrator units.

TABLE 2g. Areas of chromatogram peaks averaged over the incubation period for; A) the pooled E. carotovora varieties versus control and B) 'Russet Burbank' versus 'Norland' potatoes.

| | Ethanal | | Ethanol | | 1-Propanal/ 2-Propanone | | RT = 8.2 min | | 1-Propanol | | 1-Butanal/ 2-Butanol | | 1-Butanol | | 3-Hydroxy- 2-Butanone | | RT = 18.5 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| A | | | | | | | | | | | | | | | | | | |
| Diseased | 3.03 | 1.59 | 4.36 | 15.99 | 5.23 | 39.14 | 0.41 | 0.06 | 3.84 | 1.97 | 4.24 | 6.07 | 3.83 | 2.38 | 1.64 | 0.31 | 2.42 | 0.96 |
| Control | T | T | 3.40 | 1.91 | 4.37 | 18.41 | T | T | 1.60 | 0.41 | 3.85 | 4.85 | 3.34 | 1.91 | T | T | T | T |
| Pr > F | 0.01* | 0.06 | 0.04* | 0.12 | 0.01* | 0.06 | 0.24 | 0.09 | 0.01* | 0.13 | 0.38 | 0.71 | 0.29 | 0.84 | 0.21 | 0.42 | 0.06 | 0.21 |
| B | | | | | | | | | | | | | | | | | | |
| 'Russet Burbank' | 2.78 | 1.41 | 4.20 | 13.84 | 4.96 | 36.72 | T | T | 3.19 | 1.95 | 4.10 | 4.54 | 3.90 | 3.54 | 1.80 | 0.22 | 2.37 | 0.80 |
| 'Norland' | 2.23 | 1.22 | 4.17 | 12.94 | 5.17 | 33.76 | 0.66 | 0.76 | 3.65 | 1.46 | 4.22 | 6.94 | 3.60 | 1.26 | 0.96 | 0.27 | 1.68 | 0.77 |
| Pr > F | 0.16 | 0.19 | 0.46 | 0.76 | 0.68 | 0.78 | 0.01* | 0.01* | 0.23 | 0.59 | 0.05* | 0.01* | 0.19 | 0.05* | 0.31 | 0.75 | 0.01* | 0.41 |

*Differences significant at the 5% level.
T = Trace
[1]Total area (Log of integrator units).
[2]Percentage of total peak areas.

TABLE 2h. Chromatogram signatures of [1] *E. carotovora* var. *carotovora* (ECC), *E. carotovora* var. *atroseptica* (ECA), and control treatments [2] 'Norland' and 'Russet Burbank' potatoes.

| Treatment | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| [1] ECC | E C H J G I B M L D : afk | E C H J G I B M L D : afk |
| ECA | E C G H J D B L M : afik | E C G H J D B L M : afik |
| CO | E H C J A F G K D : bilm | E H C J A F G K D : bilm |
| [2] 'Norland' | E H C G J B D I M L F K A | E H C G J B D I M L F K A |
| 'Russet Burbank' | E C H J G B M L D I A : fk | E C H J G B M L D I A : fk |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak area.

[c] Percentage of total peak areas.

TABLE 2i. Comparison between chromatogram signatures for [1] *E. carotovora* var. *carotovora* (ECC), *E. carotovora* var. *atroseptica* (ECA), and control treatments [2] 'Norland' and 'Russet Burbank' potatoes.

| 1) | Characters Distinguishing Between Treatments[a] | | | | | |
|---|---|---|---|---|---|---|
| | ECA | | ECC | | ECA + ECC | |
| Treatment | Area[b] | PTPA[c] | Area | PTPA | Area | PTPA |
| ECA | . | . | . | . | . | . |
| ECC | HIJM | DHJM | . | . | . | . |
| CO | BEGM | * | BCEGHIJM | BCHJM | BCEGM | * |

| 2) | 'Norland' | |
|---|---|---|
| Treatment | Area | PTPA |
| 'Russet Burbank' | FHM | FHJ |

[a] Characters which differ significantly between the treatments.

[b] Log peak area.

[c] Percentage of total peak areas.

* No characteristics significantly different.

ethanol, 1-propanal/2-propanone, 1-propanol, and the unidentified compound M could be used to differentiate between the diseased and control treatments. In all cases, the differentiation was based on greater outputs by the diseased treatments. No significant PTPA differences were found for the treatments.

Differentiation between E. carotovora varieties. Generally, there were no appreciable differences in the volatile production patterns of the two E. carotovora varieties over the incubation period (Figure 2a). However, the ECC treatments appeared to produce elevated volatile levels earlier in the test period than ECA (Figure 2c).

The quantitative differences between the total volatile outputs of the E. carotovora varieties were substantial. At all points during the incubation period, the total volatile outputs for ECC were higher than ECA (Table 2b). Averaged over the entire test period, the daily volatile output for ECC was roughly double that of ECA, a difference significant at the 1% level (Table 2e).

The total daily volatile outputs for the two E. carotovora varieties followed an exponential pattern of increase over the incubation period (Figure 2c) (Table 2c). Covariant analysis of the regression equations showed that both the regression equation and its slope for ECA were significantly ($P = 0.01$) different from those for ECC (Table 2f).

All recorded chromatogram peaks were common to the profiles of both E. carotovora varieties (TABLE A). However, the number of peaks/chromatogram averaged over the incubation period was significantly ($P = 0.01$) higher in the ECC treatments (Table 2d).

The peak areas ($LOG_{10}$) and PTPA values, averaged over the entire incubation period, for the major peaks in the chromatograms of the two E. carotovora varieties are listed in Table 2j. The disease variety X potato cultivar

TABLE 2j. Areas of chromatogram peaks averaged over the incubation period for E. carotovora var. atroseptica, E. carotovora var. carotovora, and control treatments.

| | Ethanal | | Ethanol | | 1-Propanal/ 2-Propanone | | RT = 8.2 min | | -Propanol | | 1-Butanal/ 2-Butanol | | 1-Butanol | | 3-Hydroxy- 2-Butanone | | RT = 18.5 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| E. carotovora carotovora | 2.65 a* | 2.39 a | 4.67 a | 22.96 a | 5.37 a | 43.53 a | 1.97 a | 2.29 a | 3.99 a | 1.91 a | 4.66 a | 8.78 a | 4.17 a | 3.43 a | 2.06 a | 0.26 a | 3.53 a | 1.71 a |
| E. carotovora atroseptica | 2.25 a | 0.60 b | 3.96 ab | 7.30 ab | 5.05 a | 35.64 a | T a | T a | 3.67 a | 2.03 a | 3.70 b | 2.69 b | 3.40 b | 1.07 a | 1.09 a | 0.37 a | 1.04 b | 0.01 b |
| Control | T b | T b | 3.40 b | 1.91 b | 4.37 b | 18.41 a | T a | T a | 1.60 b | 0.41 a | 3.85 b | 4.85 b | 3.34 b | 1.91 a | T a | T a | T c | T b |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.

T = Trace.

1) Total area (Log of integrator units).

2) Percentage of total peak areas.

interaction was non-significant for the areas and PTPA values of all the chromatogram peaks. Consequently, the cultivars were pooled in the analysis of the disease varieties. Table 2h contains the chromatogram signatures that were developed for the mean production and PTPA values for the E. carotovora varieties (see REFERENCE TABLE B for peak identification). The peaks which could be used to differentiate between the disease varieties are summarized in Table 2i. Based upon average production levels, several compounds (1-butanal/2-butanol, 2-butanone/2-methyl-1-propanol, 1-butanol, and compound M) could be used to differentiate between the disease varieties in this test. In all cases the diagnostic difference was based on greater compound production by the ECC treatment. The PTPA values for ethanal, 1-butanal/2-butanol, 1-butanol, and compound M were different for the disease varieties. The differences in all cases were due to the greater significance of the various compounds in the ECC chromatograms.

Table 2i lists the metabolites which could be used to differentiate between each of the E. carotovora varieties and the control treatments in this experiment. The diagnostic differences for ECC vs CO could be obtained by adding the diagnostic differences for ECA vs CO to the diagnostic differences for ECC vs ECA. The differences for the average production levels and the PTPA values were, in all cases, due to greater total and relative outputs of the major volatile compounds by the disease varieties.

Differentiation between potato cultivars. There were no consistent differences in the patterns of total volatile output for the 'Russet Burbank' (R) and 'Norland' (N) tubers (Figure 2b).

Averaged over the incubation period, there was no significant ($P = 0.05$) difference in the total volatile outputs of the potato cultivars (Appendix 2a).

All recorded chromatogram peaks were common to the profiles of both cultivars (REFERENCE TABLE A). There were no consistent differences in the number of peaks/chromatogram for the cultivars (Table 2b) nor was the average number of peaks/chromatogram significantly ($P = 0.05$) different for the cultivars (Appendix 2b).

The mean areas ($LOG_{10}$) and PTPA values of the potato cultivars are presented, along with the corresponding chromatogram signatures, in Tables 2g and h (see REFERENCE TABLE B for peak identification). The metabolites, which might be used to differentiate between the 'Norland' and the 'Russet Burbank' tubers on the basis of either average peak area or PTPA values are summarized in Table 2i. Based upon average compound production levels, the compound F ('Norland' (N) significantly greater (>) than 'Russet Burbank' (R)), 1-butanal/2-butanol (N > R), and compound M (R > N) could be used to differentiate between the potato cultivars. The PTPA values for compound F (N > R) 1-butanal/2-butanol (N > R), and 1-butanol (R > N) were significantly different for the potato cultivars.

## Experiment 3: Volatile Profiles of Different Varieties of *E. carotovora*

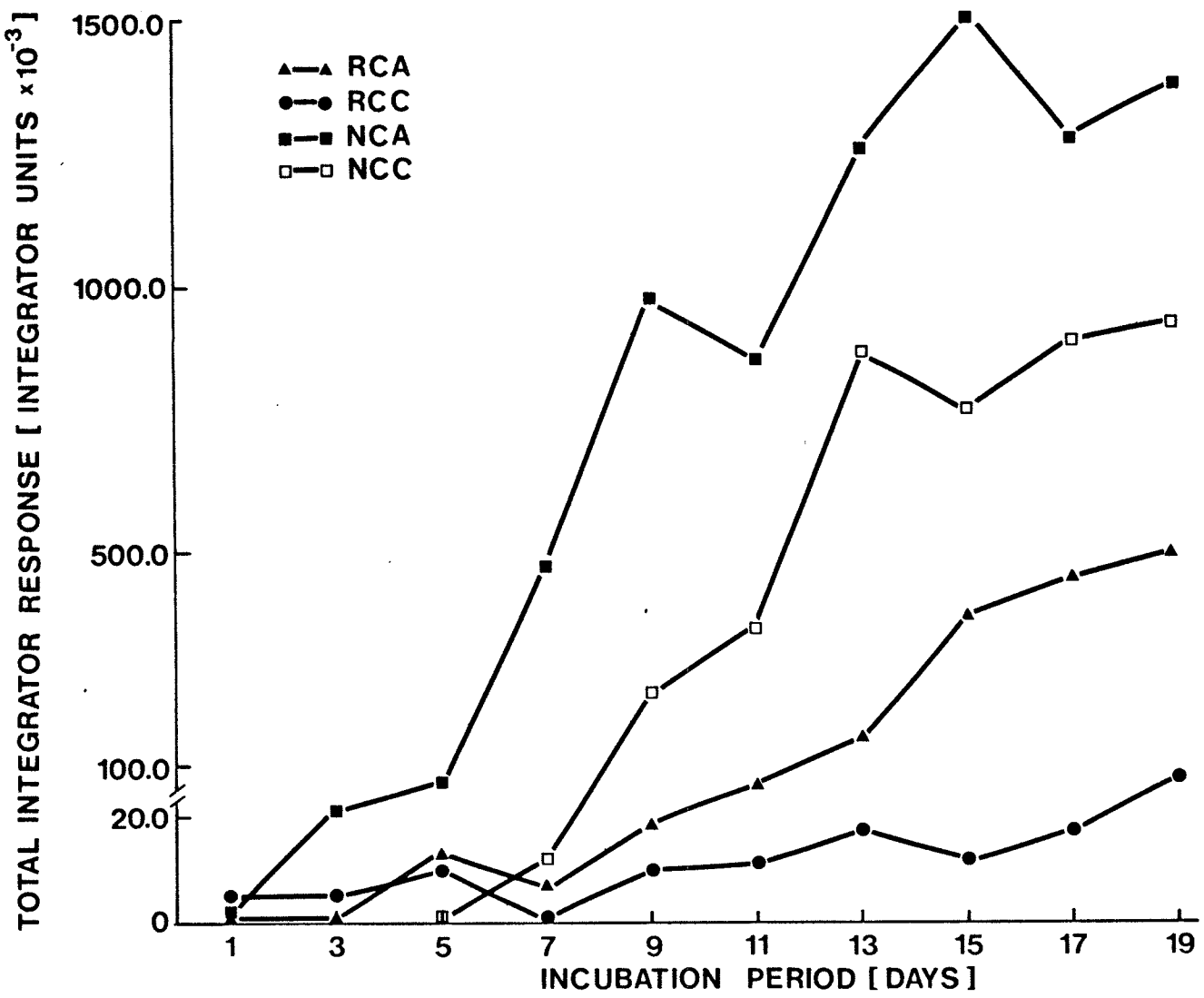
Disease summary. Three of the four control treatments (two 'Russet Burbank' and one 'Norland') began to decay within 3 to 4 days of the beginning of the experiment. *E. carotovora* var. *atroseptica* bacteria were isolated from all three of the infected controls at the end of the experiment. The control treatments were subsequently omitted from the disease analysis. Disease development amongst the infected treatments did not appear to be uniform. The RCC treatments showed no disease symptoms (visual or volatile output) until day 15. There were also obvious differences between the replicates for the other treatments.

At the termination of the experiment plating of the bacterial seepage indicated that viable *E. carotovora* bacteria were present in all disease treated samples. Although no systematic population evaluations were conducted, there appeared to be considerable differences in the pathogen population levels between the treatments and the replicates within treatments. Tests indicated that the variety specific *E. carotovora* infections had not become appreciably contaminated by the opposing variety. Plating of the bacterial seepage indicated that populations of secondary organisms were minimal in comparison with the *E. carotovora* population. *Staphylococcus*, *Clostridium*, and yeast were identified as the predominant secondary microflora.

Volatile production. In all statistical tests, the disease treatment X potato cultivar interactions were found to be non-significant and consequently were not considered in any detailed analyses.

The daily total volatile production levels for the two cultivars of potato inoculated with ECC and ECA are plotted in Figure 3a. In the RCA,

FIGURE 3a. Daily total volatile production for two cultivars of potato inoculated with E. carotovora var. carotovora and E. carotovora var. atroseptica.


TOTAL INTEGRATOR RESPONSE [ INTEGRATOR UNITS x10$^{-3}$ ]
1500.0
1000.0
500.0
100.0
20.0
0
RCA
RCC
NCA
NCC
1
3
5
7
9
11
13
15
17
19
INCUBATION PERIOD [ DAYS ]

NCA, and NCC treatments, daily volatile output increased steadily until day 15, at which time production stabilized. On the average, the volatile output of these treatments increased by a factor of 280X over the test period. The RCC treatment output levels were higher than for the other treatments during the early stages of the test. However, the volatile levels in the RCC treatment did not show the same degree of increase as occurred in the other treatments. The total volatile output of the RCC treatment only increased by 20% over the incubation period (Table 3a).

The mean daily total volatile production levels for the ECC and ECA treatments were plotted as an exponential function in Figure 3b, along with the corresponding best-fit regression lines.

The significance of the $r^2$ values indicated that the total daily volatile outputs increased in an exponential manner over the test period (Table 3b).

The average number of peaks/chromatogram increased as the diseases developed (Table 3c), although the treatment responses differed considerably.

Differentiation between E. carotovora varieties. Differentiation between the disease varieties was complicated by the variability introduced by the slow development of the RCC treatment. The pattern for disease development when 'Norland' tubers were inoculated was similar for the disease varieties (Figure 3a). The disease response of 'Russet Burbank' tubers inoculated with ECA was similar to the output pattern of both disease varieties on 'Norland' potatoes. Due to the influence of the RCC treatment, the ECA output levels were consistently greater than those for ECC (Table 3a). Averaged over the entire incubation period, the mean total volatile output of the ECA treatments was approximately 7X greater than the ECC treatments. However, due to experimental variability this difference was not significant

TABLE 3a. Relative total peak areas for 'Russet Burbank' (R) and 'Norland' (N) tubers inoculated with E. carotovora var. carotovora (ECC) and E. carotovora var. atroseptica (ECA).

| Treatment | Incubation Period, Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 |
| | % | | | | | | | | | |
| RCA | 0.1 | .1 | 1.0 | 0.8 | 1.1 | 2.1 | 8.1 | 22.0 | 26.1 | 33.4 |
| RCC | 0.5 | 0.5 | 0.8 | 0.4 | 0.7 | 0.7 | 1.2 | 1.2 | 1.9 | 5.8 |
| NCA | 0.1 | 2.1 | 4.1 | 31.1 | 61.6 | 51.3 | 90.1 | 100.0 | 85.2 | 93.4 |
| NCC | 0.1 | 0.1 | 0.1 | 0.1 | 18.4 | 24.3 | 54.4 | 28.2 | 56.6 | 55.4 |
| $\bar{X}$ CA | 0.1 | 1.1 | 2.6 | 16.0 | 31.4 | 27.0 | 49.1 | 61.0 | 55.6 | 63.4 |
| $\bar{X}$ CC | 0.3 | 0.3 | 0.4 | 0.2 | 9.6 | 12.5 | 27.8 | 14.7 | 29.2 | 30.6 |
| R | 0.3 | 0.3 | 0.9 | 0.6 | 0.9 | 1.4 | 5.1 | 11.6 | 14.0 | 19.6 |
| N | 0.1 | 1.1 | 2.1 | 15.6 | 40.0 | 32.8 | 72.2 | 64.1 | 70.9 | 74.4 |

1) Total peak area of NCA at day 15 = 100.0.

2) Value = peak area/peak area of NCA at day 15 x 100.

FIGURE 3b. Daily total volatile production for E. carotovora var. carotovora and E. carotovora var. atroseptica.

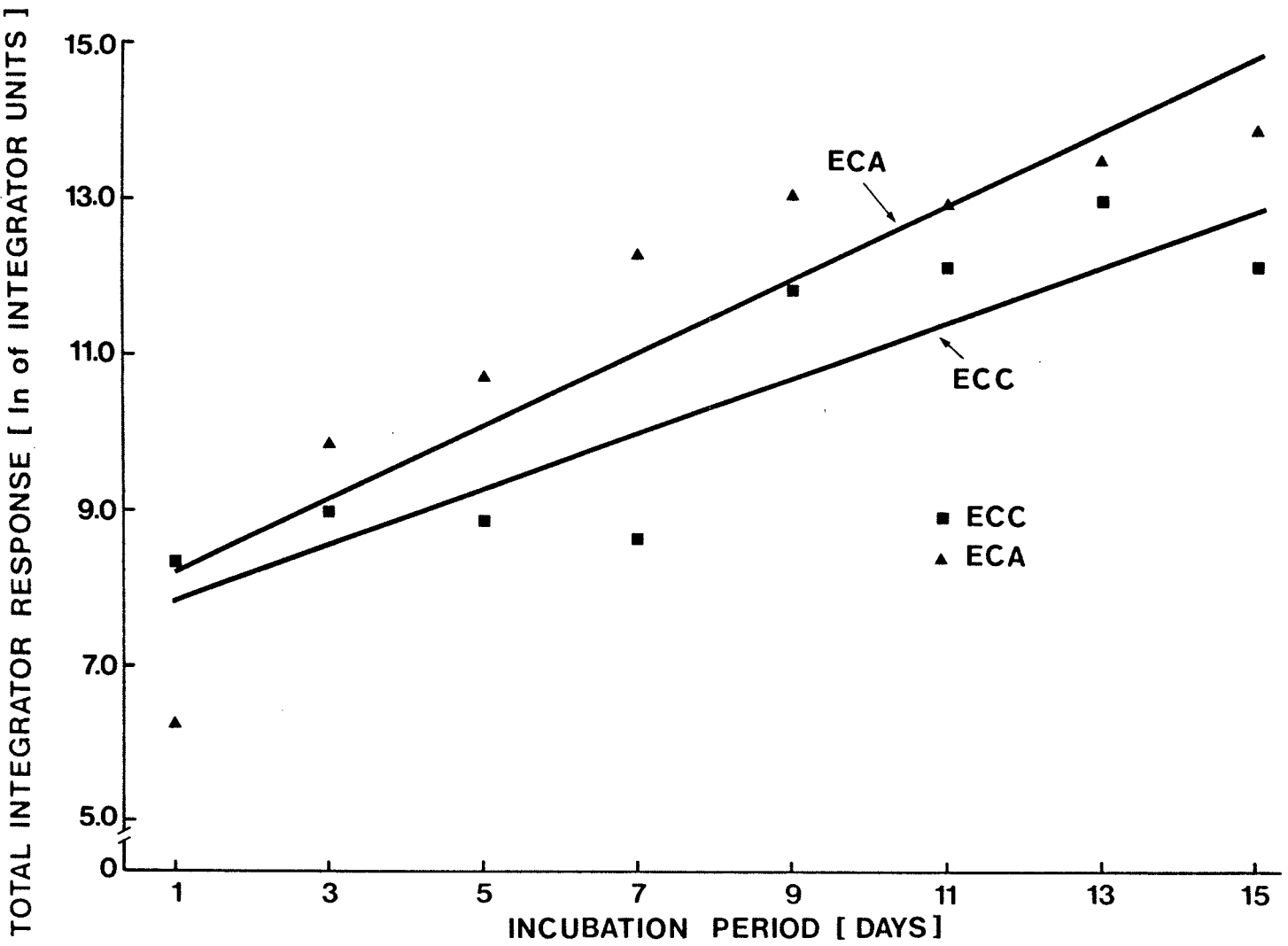

TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
15.0
13.0
11.0
9.0
7.0
5.0
0
1
3
5
7
9
11
13
15
INCUBATION PERIOD [ DAYS ]
ECA
ECC
ECC
ECA

TABLE 3b. Linear regression equations for the daily total integrator responses of E. carotovora var. carotovora and E. carotovora var. atroseptica treatments.

| Treatment | Equation | $r^2$ |
|---|---|---|
| E. carotovora var. carotovora | $Y^{1)} = e^{7.38+0.37\bar{X}^{2)}}$ | .71* |
| E. carotovora var. atroseptica | $Y = e^{7.85+0.46\bar{X}}$ | .82* |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

*Significant at the 5% level.

TABLE 3c. Mean number of peaks/chromatogram for 'Russet Burbank' (R) and 'Norland' (N) tubers inoculated with E. carotovora var. atroseptica (CA) and E. carotovora var. carotovora (CC).

| | Incubation Period, Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 |
| RCA | 1 | 1 | 1 | 1 | 5 | 6 | 7 | 8 | 10 | 11 |
| RCC | 3 | 2 | 5 | 1 | 6 | 2 | 4 | 3 | 4 | 11 |
| NCA | 1 | 1 | 4 | 6 | 11 | 11 | 11 | 11 | 10 | 11 |
| NCC | 1 | 1 | 1 | 1 | 3 | 2 | 5 | 5 | 6 | 6 |
| $\bar{X}$ CA | 1.0 | 1.0 | 4.0 | 3.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 11.0 |
| $\bar{X}$ CC | 2.0 | 1.5 | 3.0 | 1.0 | 4.5 | 2.0 | 4.5 | 4.0 | 5.0 | 8.5 |
| $\bar{X}$ R | 2.0 | 1.5 | 4.5 | 1.0 | 5.5 | 4.0 | 5.5 | 5.5 | 7.0 | 11.0 |
| $\bar{X}$ N | 1.0 | 1.0 | 2.5 | 3.5 | 7.0 | 6.5 | 8.0 | 8.0 | 8.0 | 8.5 |

at the 5% level (Appendix 3a). The disease X cultivar interaction was found to be non-significant despite the minimal response of the RCC treatment.

Covariant analysis of the regression equation derived for the mean daily total peak areas showed no significant difference (P = 0.05) in the slopes or the general regression equations for the two E. carotovora varieties (Table 3d).

All recorded chromatogram peaks were common to both E. carotovora varieties (REFERENCE TABLE A). The number of peaks/chromatogram on a given day was consistently lower in the ECC treatments (Table 3c), however, the number of peaks/chromatogram when averaged over the incubation period was not significantly different (P = 0.05) (Appendix 3b).

The mean areas ($LOG_{10}$) and PTPA values, averaged over the incubation period, for the major peaks in the disease variety chromatograms are presented in Table 3e. Table 3f contains the chromatogram signatures that were developed for the peak areas and PTPA values (see REFERENCE TABLE B for peak identification). The metabolites which, according to the test statistics in Table 3e, could potentially be used to differentiate between the E. carotovora varieties' volatile profiles are summarized in Table 3g. On the basis of average compound production levels, ethanol (ECA > ECC), 1-propanal/2-propanone (ECA > ECC), and the unidentified compound K (ECC > ECA) could be used to differentiate between the E. carotovora varieties. The PTPA values for 1-propanal/2-propanone (ECA > ECC) differed significantly for the two E. carotovora varieties.

Differentiation between potato cultivars. Analysis of cultivar differences was also complicated by the minimal disease response of the RCC treatment. The total daily volatile production levels indicated that both varieties of E. carotovora developed more slowly in the 'Russet Burbank' tubers (Figure 3a) (Table 3a). Although the mean relative peak areas for the 'Russet

TABLE 3d. Analysis of covariance for the linear regression equations calculated for the daily total responses[1)] of E. carotovora var. carotovora and E. carotovora var. atroseptica treatments.

| Pairwise comparison | F Test for slope | F Test for similarity between lines |
|---|---|---|
| E. carotovora var. carotovora | | |
| vs | $0.45^{N.S.}$ | $3.82^{N.S.}$ |
| E. carotovora var. atroseptica | | |

N.S. Difference not significant at the 5% level.

1) Ln of integrator units.

TABLE 3e. Areas of chromatogram peaks averaged over the incubation period for two cultivars of potato inoculated with *E. carotovora* var. *carotovora* (ECC) and *E. carotovora* var. *atroseptica* (ECA).

| | Ethanal | | Ethanol | | 1-Propanal/ 2-Propanone | | RT = 8.2 min | | 1-Propanol | | 1-Butanal/ 2-Butanol | | 1-Butanol | | 3-Hydroxy- 2-Butanone | | RT = 18.5 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| ECC | 1.67 a* | 0.60 a | 1.42 b | 2.24 a | 1.42 b | 0.86 b | 0.73 a | 0.41 a | 1.90 a | 1.46 a | 0.53 a | 1.58 a | 2.06 a | 5.99 a | 1.06 a | 0.78 a | 0.77 a | 5.15 a |
| ECA | 3.32 a | 1.52 a | 4.39 a | 13.98 a | 4.61 a | 32.02 a | 2.85 a | 1.02 a | 2.98 a | 1.66 a | 2.90 a | 0.03 a | 4.49 a | 11.87 a | 2.79 a | 0.30 a | 2.11 a | 1.40 a |
| 'Russet Burbank' | 1.47 a | 1.00 a | 2.81 a | 5.01 a | 2.76 a | 3.60 a | 0.96 a | 0.55 a | 0.94 a | 0.32 a | 0.71 a | 14.03 a | 1.96 a | 3.00 a | T a | T a | 2.01 a | 8.22 a |
| 'Norland' | 3.06 a | 0.98 a | 2.60 a | 8.96 a | 2.81 a | 22.18 a | 2.14 a | 0.76 a | 3.43 a | 2.47 a | 2.17 a | 0.39 a | 3.97 a | 12.64 a | 3.15 a | 1.86 a | 0.84 a | 0.04 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.

T = Trace.

1) Log of integrator areas.

2) Percentage of total peak areas.

TABLE 3f. Chromatogram signatures of [1] *E. carotovora* var. *carotovora* (ECC) and *E. carotovora* var. *atroseptica* (ECA) and [2] 'Norland' and 'Russet Burbank' potatoes.

| Treatment | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| [1] ECC | J I G B K E C L D M F A : h | J I G B K E C L D M F A : h |
| ECA | E J C B G H L F I M D : ak | E J C B G H L F I M D : ak |
| [2] 'Norland' | J G L B I E C H F D M A K | J G L B I E C H F D M K |
| 'Russet Burbank' | E C J K M B I D G H F L : a | E C J K M B I D G H F L : a |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak area.

[c] Percentage of total peak area.

TABLE 3g. Comparison between chromatogram signatures for [1] *E. carotovora* var. *carotovora* (ECC) and *E. carotovora* var. *atroseptica* (ECA) and [2] 'Norland' and 'Russet Burbank' potatoes.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | ECA | |
| | Area[b] | PTPA[c] |
| ECC | CEK | E |
| | 'Norland' | |
| | Area | PTPA |
| 'Russet Burbank' | * | * |

[a] Characters which differ significantly between the treatments.

[b] Log peak area.

[c] Percentage of total peak area.

* No characters significantly different.

Burbank' tubers were consistently well below those for 'Norland' (Table 3a), there was no significant (P = 0.05) difference between the two cultivars when the total volatile outputs were averaged over the entire incubation period (Appendix 3a).

All recorded chromatogram peaks were common to the volatile profiles of both potato cultivars (REFERENCE TABLE A). There were no consistent differences in the chromatogram complexity of the cultivars (Table 3c), nor was the average number of peaks/chromatogram significantly different (P = 0.05) for the potato cultivars (Appendix 3b).

The mean areas ($LOG_{10}$) and PTPA values of the two potato cultivars are presented, along with the corresponding chromatogram signatures in Tables 3e and f (see REFERENCE TABLE B for peak identification). The cultivars could not be differentiated on the basis of either the mean area or the PTPA values of any of the volatiles identified in this experiment.

Experiment 4: Volatile Profiles of Diseased and Healthy Tubers

Disease summary. The soft-rot infection appeared to develop in a uniform manner within the replicates of the E. carotovora inoculated treatment (EMX). By day 3 there was obvious pitting of the tuber surfaces and the beginning of seepage from the inoculation points. By day 6, tuber breakdown had progressed to the point where seepage prevented further sampling. The individual tubers within each bag tended to decay at approximately the same rate. The controls (CO) remained sound throughout the incubation period.

Tests at the end of the experiment showed that, in all disease replicates, both varieties of E. carotovora were present in the bacterial seepage. Populations of secondary microorganisms were minimal in comparison to the E. carotovora populations. Yeasts were the dominant organisms in the secondary microflora.

Volatile production. The schematic chromatograms representing peak areas and percentages of total peak area (PTPA) (Figure 4a, top section) illustrate a number of the changes that occurred in the potatoes' volatile profiles during the progression of an E. carotovora infection. Volatile output, as measured by both the number of peaks in each days' chromatogram (Table 4a) and the total amounts of the various components produced, increased very rapidly during the progression of the disease (Figure 4a). Over the 5 day incubation period, the total daily volatile outputs for the EMX treatments increase 66X over the volatile production levels on day 1 (Table 4b).

The peaks identified in the chromatograms are listed in REFERENCE TABLE C. In many cases, the individual metabolites did not respond identically over the duration of the experiment. For example, ethanol (Peak C) appeared at relatively minor levels in the early chromatograms. In the subsequent chromatograms, ethanol became the predominant peak. Its output levels kept pace

FIGURE 4a. Areas and PTPA* values for chromatogram peaks at three stages of a mixed E. carotovora infection.

*Percentage of total peak areas.

Peak A = Methanol

B = Ethanal

C = Ethanol

D = ?

E = 1-Propanol

F = 1-Butanal/2-Butanol/2-Butanone/2-Methyl-1-Propanol (C-4 Compounds)

G = 1-Butanol

H = ?

I = 3-Hydroxy-2-Butanone

J = ?

Y Axis = Peak areas

X Axis = Chromatogram peaks

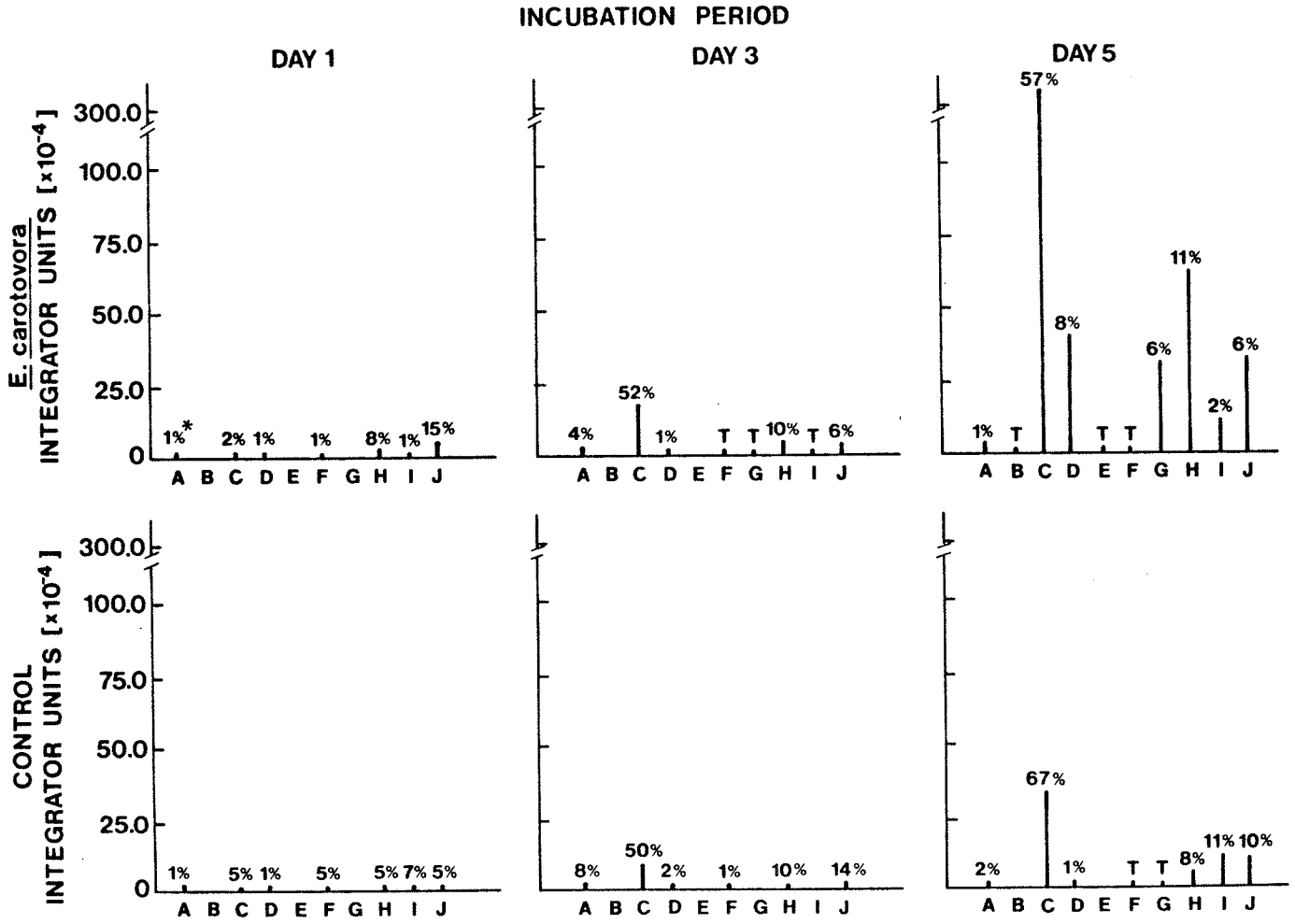

INCUBATION PERIOD
DAY 1
DAY 3
DAY 5
E. carotovora
INTEGRATOR UNITS [×10⁻⁴]
CONTROL
INTEGRATOR UNITS [×10⁻⁴]
300.0
100.0
75.0
50.0
25.0
0
1%*
2% 1%
1%
8% 1%
15%
4%
52%
1%
T T
10%
T
6%
1% T
57%
8%
T T
6%
11%
2%
6%
1%
5% 1%
5%
5% 7% 5%
8%
50%
2%
1%
10%
14%
2%
67%
1%
T T
8%
11% 10%
A B C D E F G H I J

TABLE 4a. Number of peaks/chromatogram for a mixed *E. carotovora* infection.

| | Incubation Period (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *E. carotovora* | 14 | 14 | 15 | 16 | 16 |
| Control | 10 | 6 | 8 | 9 | 11 |

TABLE 4b. Relative total peak areas[1] for a mixed *E. carotovora* infection.

| | Incubation Period (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | % | | | | |
| *E. carotovora* | 1.2[2] | 1.4 | 5.0 | 49.7 | 100.0 |
| Control | 0.9 | 1.4 | 4.1 | 10.0 | 10.1 |

[1] Total peak area of *E. carotovora* at 5 days = 100.0%.

[2] Values = peak area/peak area *E. carotovora* day 5.

with the general increase in volatile production as reflected by the relatively stable PTPA values. By contrast, the $C_4$ compounds (Peak F) were present throughout the infection, however, their production levels did not increase significantly over the duration of the test period. Concequently, the PTPA values for peak F declined to trace amounts.

The daily volatile outputs of the EMX treatments, when plotted as an exponential function (Figure 4b), showed a very good fit (P = 0.01) to the exponential regression equation (Table 4c).

The volatile profiles for the control treatments (CO) were clearly distinguishable from those of the diseased treatments (Figure 4a). Although there was a considerable overlap in the compounds detected in the treatments' volatile profiles, several compounds (i.e., 2-propanol, 1-propanal/2-propanone, 1-propanol, and two unidentified compounds with retention times of 7.1 and 14.1 min) present at significant levels in the EMX treatments could not be detected in the controls (REFERENCE TABLE C).

At all stages during the incubation period, the number of peaks/chromatogram for the control treatments was consistently lower than for the EMX treatments (Table 4a). When averaged over the entire incubation period, this difference was significant at the 1% level (Table 4d).

The total daily volatile output of the controls increased by a factor of 9X over the incubation period (Figure 4b; Table 4b). At most points, the total volatile output of the diseased treatments was greater than in the controls (Table 4b). Averaged over the entire test period, the EMX treatments' volatile output was approximately 8X greater than that of the control; a difference significant at the 1% level (Table 4e).

The $r^2$ value for the regression equation derived for the daily volatile output of the control treatment indicates that the data followed an exponential production pattern (P = 0.01) over the incubation period (Table 4c).

REFERENCE TABLE C. Volatile peaks occurring in bag experiments (4-10).

| Retention time (min.) | Compound identity | *E. carotovora* var. *carotovora* | *E. carotovora* var. *atroseptica* | *C. sepedonicum* | Control | Russet Burbank | Norland |
|---|---|---|---|---|---|---|---|
| 2.5 | Methanol | * | * | * | * | * | * |
| 3.1 | Ethanal | * | * | | * | * | * |
| 4.4 | Ethanol | * | * | * | * | * | * |
| 5.9 | 2-Propanol | * | * | * | * | * | * |
| 6.5 | 1-Propanal/ 2-Propanone | * | * | | * | * | * |
| 7.1 | ? | * | * | | * | * | * |
| 8.1 | 1-Propanol | * | * | * | * | * | * |
| 9.5-10.1 | 2-Butanol/ 2-Butanone 1-Butanal 2-Methyl-1-Propanol | * | * | * | * | * | * |
| 11.8 | 1-Butanol | * | * | * | * | * | * |
| 12.7 | ? | * | * | | * | * | * |
| 14.1 | ? | * | * | | | * | * |
| 14.6 | 3-Hydroxy-2-Butanone | * | * | * | * | * | * |
| 15.6 | ? | | | * | | * | NA |
| 16.3 | ? | * | * | | | * | * |
| 17.8 | ? | * | * | * | * | * | * |

* = Detected in treatment.

FIGURE 4b. Daily total volatile production and corresponding best-fit lines for a mixed E. carotovora infection and control treatments.

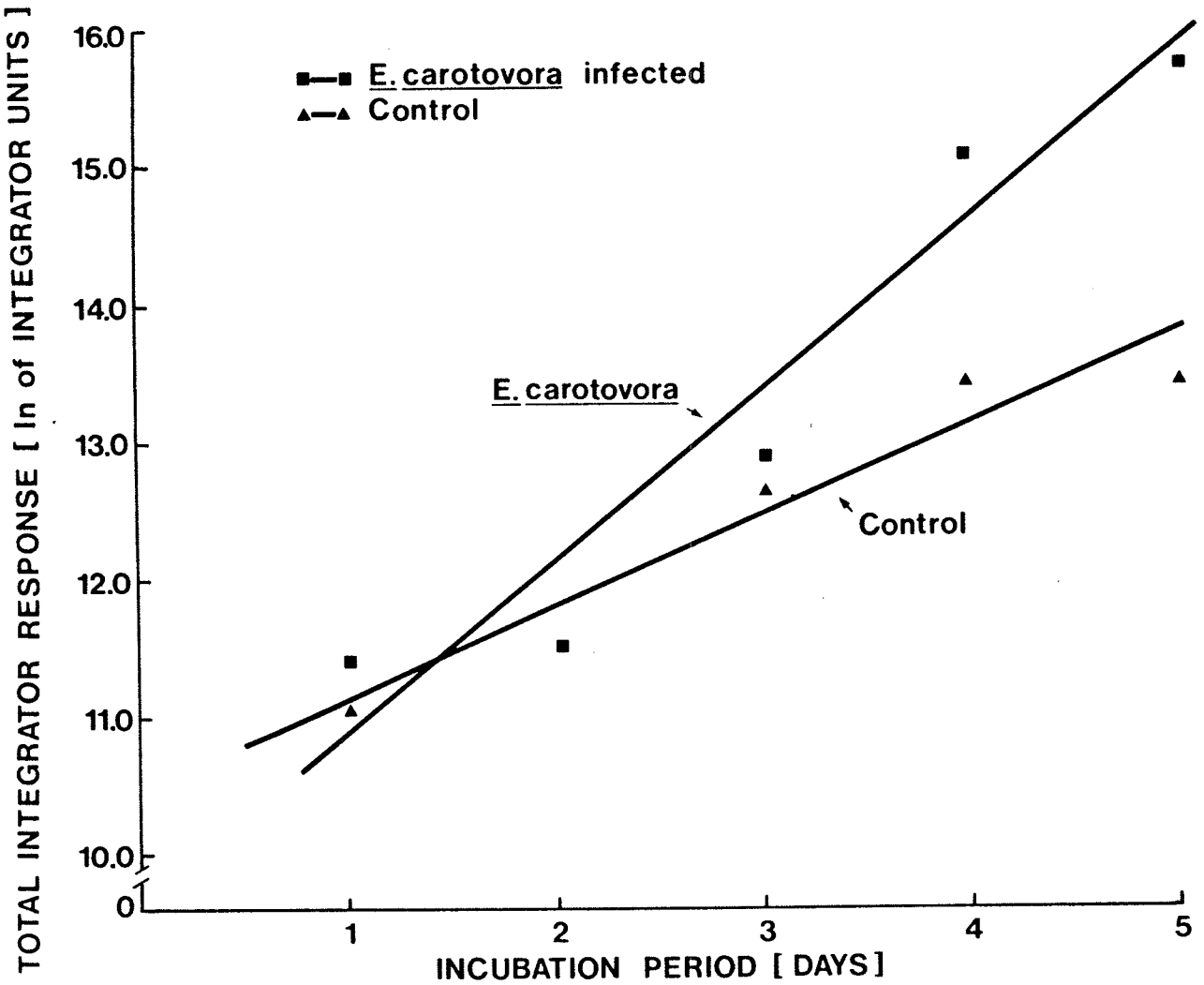

E. carotovora infected
Control
E. carotovora
Control
TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
16.0
15.0
14.0
13.0
12.0
11.0
10.0
0
1
2
3
4
5
INCUBATION PERIOD [ DAYS ]

TABLE 4c. Linear regression equations for the daily total integrator responses of a mixed E. carotovora (EMX) infection.

| Treatment | Equation | $r^2$ |
|---|---|---|
| EMX | $Y^{1)} = e^{9.66+1.24\bar{X}^{2)}}$ | .85** |
| Control | $Y = e^{10.57+0.63\bar{X}}$ | .76** |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

** Significant at the 1% level.

TABLE 4d. Pairwise comparison of mean numbers of peaks/chromatogram averaged over the incubation period for a mixed E. carotovora (EMX) infection.

| Pairwise comparison | Mean number peaks/chromatogram | t Value |
|---|---|---|
| EMX | 10.61 | 4.64** |
| + | | |
| Control | 6.34 | |

** Significant at the 1% level.

TABLE 4e. Comparison of means of total peak areas[1] averaged over the incubation period for a mixed *E. carotovora* (EMX) infection.

| Pairwise comparison | Mean total integrator response | t Value |
|---|---|---|
| EMX | 6.322 | |
| vs | | 7.68** |
| Control | 5.439 | |

**Values significantly different at the 1% level.

1) Log of sum of all individual peak areas.

Analysis of covariance tests indicated that both the regression equation and its slope for the controls were significantly ($P = 0.01$) different from the equation for the EMX treatment (Table 4f).

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the EMX infected and control treatments are listed in Table 4g, with the corresponding chromatogram signatures listed in Table 4h (peaks identified in REFERENCE TABLE D). Table 4i summarizes the metabolites which, according to the test statistics in Table 4g, could potentially be used to differentiate between the volatile profiles of the E. carotovora infected treatments and the controls. Based on average production levels, a number of metabolites (ethanol, compound D, 1-propanol, the C-4 compounds, and compound H) could be used to differentiate between diseased and control treatments. In all cases, the difference was due to greater volatile production by the EMX infected treatment. No significant differences were found in the PTPA values of the EMX and control treatments.

TABLE 4f. Analysis of covariance for the linear regression equations calculated for the daily total integrator responses[1)] of a mixed E. carotovora (EMX) infection.

| Pairwise comparison | F Test for slopes | F Test for similarity between lines |
|---|---|---|
| EMX + Control | 12.25** | 9.94** |

** Difference significant at the 1% level.

1) Ln of integrator units.

TABLE 4g. Areas of chromatograph peaks averaged over the incubation period for a mixed E. carotovora infection.

| | Methanol | | Ethanal | | Ethanol | | RT = 7.1 min | | 1-Propanol | | C-4 pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| E. carotovora | 4.50 | 3.53 | 1.05 | 0.06 | 5.78 | 37.65 | 4.55 | 2.51 | 3.27 | 0.01 | 3.68 | 1.46 | 4.60 | 2.09 | 5.28 | 11.11 | 3.29 | 2.11 | 4.83 | 8.57 |
| Control | 1.77 | 11.17 | T | T | 4.43 | 44.50 | 3.03 | 0.50 | T | T | T | T | 2.85 | 0.17 | 4.44 | 6.50 | 3.39 | 1.50 | 4.69 | 8.33 |
| Pr > F. | 0.30 | 0.30 | - | - | 0.02* | 0.36 | 0.03* | 0.34 | - | - | - | - | 0.01* | 0.29 | 0.01* | 0.10 | 0.96 | 0.75 | 0.75 | 0.94 |

T = Trace

1) Total area (Log of integrator units).

2) Percentage of total peak areas.

* Difference significant at the 5% level.

REFERENCE TABLE D. Tentative identification of chromatogram peaks listed in chromatogram signatures.

Bag Experiments (EXPT 4-10)

A = Methanol

B = Ethanal

C = Ethanol

D = Unidentified (RT = 7.1 min)

E = 1-Propanol

F = $C_4$-Compounds

G = 1-Butanol

H = Unidentified (RT = 12.7 min)

I = 3-Hydroxy-2-Butanone

J = Unidentified (RT = 16.3 min)

K = Unidentified (RT = 17.8 min)

TABLE 4h. Chromatogram signatures for a mixed E. carotovora infection and non-infected control treatment.

| Treatment | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| E. carotovora | C H K G D A F E B J | C H K G D A F I E B J |
| Control | K C H I D G A E F : bj | C A K H I D E G F : bj |

a) Peak significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 4i. Comparisons between the chromatogram signatures for a mixed E. carotovora infection and non-infected control treatments.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | E. carotovora | |
| | Area[b] | PTPA[c] |
| Control | CDEFH | * |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

Experiment 5: Volatile Profiles of Different Varieties of E. carotovora.

Disease summary. Both times that this experiment was run, contamination of the control treatments was a constant problem. In Trial 1, E. carotovora var. atroseptica bacteria caused the decay of one of the wounded controls (CO) and one of the non-wounded controls (CX). In Trial 2, both non-wounded controls and one of the wounded controls were found to have been infected with E. carotovora var. carotovora and E. carotovora var. atroseptica. The non-wounded treatment in Trial 2 was subsequently dropped from the experimental analysis. In both trials, the data from the remaining contaminated treatments was included in the experimental analysis.

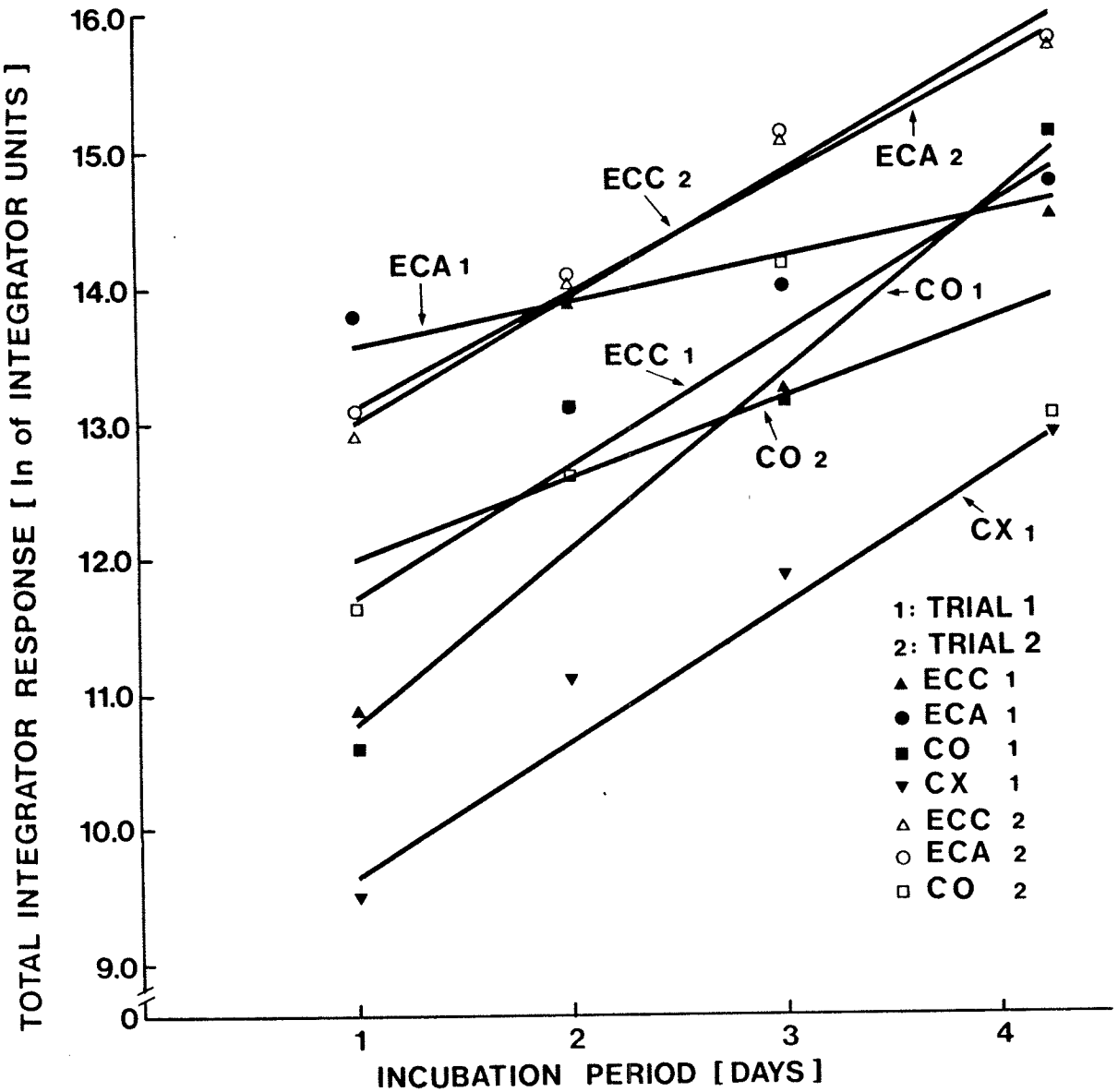
In the E. carotovora inoculated treatments, disease development within the experimental replicates was relatively uniform. In both trials, the degree of tuber decay by day 5 of the incubation period was sufficient to interfere with any further volatile sampling.

E. carotovora bacteria were present in the bacterial seepage of all infected treatments at the end of the incubation period. Tests indicated that one of the ECC treatments had become contaminated with ECA bacteria in Trial 2. This replicate was dropped from the experimental analysis.

In both trials, populations of secondary microflora in the bacterial seepage were relatively small in comparison to the E. carotovora populations. In Trial 1, yeast, E. coli, and Staphylococcus dominated the secondary microflora. In Trial 2, only yeasts were found in appreciable numbers.

Volatile production. The daily total volatile outputs of the experimental treatments are plotted as an exponential function in Figure 5a, along with the corresponding best-fit regression lines. In Trial 1, the daily total volatile output data of the ECC ($P = 0.01$), CO ($P = 0.01$), and CX ($P = 0.05$)

FIGURE 5a. Daily total volatile production and corresponding best-fit regression lines for E. carotovora varieties (ECA and ECC), wounded control (CO), and non-wounded controls (CX).


TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
0
1
2
3
4
INCUBATION PERIOD [ DAYS ]
ECA 1
ECC 2
ECA 2
CO 1
ECC 1
CO 2
CX 1
1: TRIAL 1
2: TRIAL 2
▲ ECC 1
● ECA 1
■ CO 1
▼ CX 1
△ ECC 2
○ ECA 2
□ CO 2

treatments fit the exponential regression equations given in Table 5a. The ECA data did not show a significant (P = 0.05) degree of fit to the regression equation, primarily due to the unusually high volatile outputs recorded on day 1. In Trial 2, the volatile production data for both the ECC and ECA treatments showed a very good fit (P = 0.01) to the derived regression equations. The CO data did not show a significant degree of fit (P = 0.05) to the regression equation primarily due to the variance introduced by the E. carotovora contamination of one of the treatments.

In Trial 1, the total volatile outputs of the disease treatments did not develop similarly over the duration of the test period. In the ECC treatment, the total volatile production increased steadily, with the final output being 33 times greater than the levels on day 1. In the ECA treatment, volatile levels on day 1 were unusually high. The final volatile production level was similar to that recorded for the more typical ECC treatment (Figure 5a). The uncontaminated replicate of the CO treatment had a relatively stable low level of volatile production over the incubation period. The infected replicate had a volatile production pattern that closely resembled the inoculated treatments. In the non-wounded control, the data presented in Figure 5a is again an average for one healthy and one E. carotovora contaminated replicate.

In Trial 2, the volatile outputs of the disease treatments were very similar. In both ECC and ECA, total volatile production increased steadily over the test period. The initial and final outputs of the disease treatments were very similar as was the degree of increase in volatile production over the incubation period (approximately 15 times). In the wounded control, volatile production was erratic due to the E. carotovora contamination.

Differentiation between disease treatments. Table 5b contains the test statistics for the covariant analyses of the total volatile production

TABLE 5a. Linear regression equations for the daily total integrator responses of the E. carotovora varieties (ECA and ECC), wounded (CO), and non-wounded (CX) controls.

| Treatment | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | Equation | $r^2$ | Equation | $r^2$ |
| ECA | $Y^{1)} = e^{13.12+0.31\bar{X}^{2)}}$ | $0.06^{N.S.}$ | $Y = e^{12.06+0.96\bar{X}}$ | $0.92^{**}$ |
| ECC | $Y = e^{10.60+1.00\bar{X}}$ | $0.68^{**}$ | $Y = e^{12.38+0.88\bar{X}}$ | $0.90^{**}$ |
| CO | $Y = e^{9.41+1.46\bar{X}}$ | $0.87^{**}$ | $Y = e^{11.40+0.58\bar{X}}$ | $0.18^{N.S.}$ |
| CX | $Y = e^{8.67+1.06\bar{X}}$ | $0.61^{*}$ | NA | |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

** Significant at the 1% level.

* Significant at the 5% level.

N.S. Not significant at the 5% level.

TABLE 5b. Analysis of covariance for the linear regression equations calculated for the daily total integrator responses[1)] of the E. carotovora varieties (ECA and ECC), wounded (CO), and non-wounded (CX) controls in two experimental trials.

| | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| Pairwise comparisons | F Test for slopes | F Test for similarity of lines | F Test for slopes | F Test for similarity of lines |
| ECA vs ECC | $28.13^{**}$ | $0.99^{N.S.}$ | $0.89^{N.S.}$ | $1.16^{N.S.}$ |
| ECA vs CO | $5.06^{*}$ | $1.91^{N.S.}$ | $1.06^{N.S.}$ | $28.86^{**}$ |
| ECC vs CO | $2.97^{N.S.}$ | $0.88^{N.S.}$ | $1.62^{N.S.}$ | $27.69^{**}$ |
| ECA vs CX | $2.35^{N.S.}$ | $18.21^{**}$ | NA | NA |
| ECC vs CX | $10.65^{**}$ | $39.99^{**}$ | NA | NA |
| CO vs CX | $0.90^{N.S.}$ | $13.88^{**}$ | NA | NA |

1) Ln of integrator units.

** Difference significant at the 1% level.

* Difference significant at the 5% level.

N.S. Difference not significant at the 5% level.

regression equations derived for the experimental treatments. In Trial 1, comparisons of the treatments were complicated by the contamination of the control treatments and by the erratic volatile production levels of the ECA treatment. The regression equations for the ECC and ECA treatments were similar to those for the wounded control treatments. The ECA treatment could be differentiated from the wounded controls on the basis of the regression equation slopes ($P = 0.05$) and from the non-wounded control on the basis of the overall regression equation ($P = 0.01$). The ECC regression equation could not be differentiated from the wounded control ($P = 0.05$), but both the regression equations and their slopes ($P = 0.01$) were significantly different when the ECC treatment was compared with the non-wounded control (CX). The regression equation for the CO treatment was significantly ($P = 0.01$) different from the CX equation, however, the slopes were not distinguishable ($P = 0.05$).

In Trial 2, the regression equations for both disease treatments were significantly ($P = 0.01$) different from the equation for the CO treatment, however, the slopes were found to be similar ($P = 0.05$).

In Trial 1, the average daily volatile output of the diseased treatments (ECC and ECA) was approximately three times greater than the CO treatments (Table 5c). However, this difference was not significant at the 5% level. The diseased treatments produced, on average, seven times the volatile output of the CX treatments; in both cases this difference was significant at the 1% level. The average volatile output of the CO treatment was not significantly different from the non-wounded controls ($P = 0.05$).

In Trial 2, the average daily volatile outputs of the diseased treatments (ECC and ECA) was approximately seven times greater than the CO treatment. In both cases, this difference was significant at the 1% level (Table 5c).

TABLE 5c. Pairwise comparisons of means of the total of peak areas[1] averaged over the incubation period for the E. carotovora varieties (ECA and ECC) and control treatments (CO and CX).

| Pairwise comparison | Trial # | Trmt | Mean total integrator response | t Value |
|---|---|---|---|---|
| ECA vs ECC | 1 | ECA | 6.239 | 0.91 N.S. |
| | | ECC | 6.405 | |
| | 2 | ECA | 6.568 | 0.01 N.S. |
| | | ECC | 6.566 | |
| ECA vs CO | 1 | ECA | 6.239 | 1.52 N.S. |
| | | CO | 5.872 | |
| | 2 | ECA | 6.568 | 4.83** |
| | | CO | 5.697 | |
| ECC vs CO | 1 | ECC | 6.405 | 2.21 N.S. |
| | | CO | 5.872 | |
| | 2 | ECC | 6.566 | 4.81** |
| | | CO | 5.697 | |
| ECA vs CX | 1 | ECA | 6.239 | 3.11** |
| | | CX | 5.489 | |
| | 2 | ECA | NA | |
| | | CX | | |
| ECC vs CX | 1 | ECC | 6.405 | 3.80** |
| | | CX | 5.489 | |
| | 2 | ECC | NA | |
| | | CX | | |
| CO vs CX | 1 | CO | 5.872 | 1.32 N.S. |
| | | CX | 5.489 | |
| | 2 | CO | NA | |
| | | CX | | |

**Values significantly different at the 1% level.

N.S. Values not significantly different at the 5% level.

1) Log of sum of all individual peak areas.

In Trial 1, the average number of peaks/chromatogram for the diseased treatments was significantly greater than the values for both the CO ($P = 0.01$) and CX ($P = 0.01$) treatments (Table 5d). The CO and CX treatments did not produce significantly different numbers of peaks/chromatogram ($P = 0.05$). In Trial 2, the number of peaks/chromatogram in the ECA treatment was significantly ($P = 0.01$) greater than for the CO treatment (Table 5d). The ECC and CO treatments did not yield significantly ($P = 0.05$) different numbers of peaks/chromatogram.

In Trial 1, a number of compounds (2-propanol, 1-propanal/2-propanone, and two unidentified compounds; RT = 7.1 and 14.1 min) were present in the diseased treatment and in those controls (CO and CX) which had become contaminated with E. carotovora but were not detected in the uncontaminated controls (Table 5c). In Trial 2, 2-propanol and two compounds (RT = 14.1 and 15.6 min) were present in the diseased treatments, but not in the wounded controls.

The mean areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the disease treatments are listed in Table 5e and the corresponding chromatogram signatures are listed in Table 5f (see REFERENCE TABLE D for peak identification). The metabolites which, according to the test statistics in Table 5e could be used to differentiate between the disease treatments are summarized in Table 5g. When comparing the peak area and PTPA values of the ECA and CO treatments, all differentiations were based on the greater total and relative volatile outputs of the ECA treatment.

When comparing the peak area values of the ECC and CO treatments, all differentiations were also based on the greater total volatile outputs of the diseased treatment. For the PTPA values, the two experimental trials produced similar data for ethanol (CO > ECC), the C-4 compounds (ECC > CO),

TABLE 5d. Pairwise comparisons of mean numbers of peaks/chromatogram averaged over the incubation period for the E. carotovora varieties (ECC and ECA), wounded (CO), and non-wounded (CX) control treatments.

| Pairwise comparison | Trial # | Trmt. | Mean number of peaks/chromatogram | t Value |
|---|---|---|---|---|
| ECA vs ECC | 1 | ECA | 9.21 | $1.69^{N.S.}$ |
| | | ECC | 8.04 | |
| | 2 | ECA | 9.72 | $2.09^{N.S.}$ |
| | | ECC | 8.48 | |
| ECA vs CO | 1 | ECA | 9.21 | $4.89^{**}$ |
| | | CO | 4.63 | |
| | 2 | ECA | 9.72 | $3.84^{**}$ |
| | | CO | 7.30 | |
| ECC vs CO | 1 | ECC | 8.04 | $3.61^{**}$ |
| | | CO | 4.63 | |
| | 2 | ECC | 8.48 | $1.87^{N.S.}$ |
| | | CO | 7.30 | |
| ECA vs CX | 1 | ECA | 9.21 | $3.83^{**}$ |
| | | CX | 5.60 | |
| | 2 | ECA | NA | |
| | | CX | | |
| ECC vs CX | 1 | ECC | 8.04 | $2.55^{*}$ |
| | | CX | 5.60 | |
| | 2 | ECC | NA | |
| | | CX | | |
| CO vs CX | 1 | CO | 4.63 | $0.89^{N.S.}$ |
| | | CX | 5.60 | |
| | 2 | CO | NA | |
| | | CX | | |

$^{**}$Values significantly different at the 1% level.

$^{*}$Values significantly different at the 5% level.

$^{N.S.}$Values not significantly different at the 5% level.

TABLE 5e. Areas of chromatogram peaks averaged over the incubation period for E. carotovora varieties (ECA and ECC) and wounded (CO) and non-wounded (CX) control treatments.

| | Methanol | | Ethanal | | Ethanol | | RT = 7.1 min | | C-4 | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| Trial 1 | | | | | | | | | | | | | | | | | | |
| ECA | 5.34 a* | 8.35 a* | 0.80 a | T a | 5.76 a | 54.15 a | 3.66 ab | 0.30 b | 3.98 a | 0.55 b | 3.83 a | 0.80 a | 5.07 b | 4.50 b | 3.81 a | 0.50 b | 5.00 a | 5.40 a |
| ECC | 5.32 a | 10.20 a | 0.87 a | 0.05 a | 5.56 ab | 44.85 b | 3.86 a | 0.20 b | 4.37 ab | 1.45 a | 3.67 a | 0.20 a | 5.53 a | 8.60 a | 3.90 a | 0.55 b | 5.11 a | 8.25 a |
| CO | 5.10 a | 9.75 a | 0.79 a | T a | 5.56 ab | 58.50 a | 3.60 ab | 0.90 b | 3.44 bc | TR b | 3.02 a | T a | 4.55 c | 2.13 b | 3.01 b | 0.25 b | 4.55 a | 3.00 a |
| CX | 4.01 b | 8.00 a | T a | T a | 3.99 b | 20.75 c | 3.29 b | 7.00 a | 2.63 c | 1.25 ab | T b | T a | 3.54 d | 7.5 ab | 3.34 cd | 6.25 a | 4.16 a | 15.00 a |
| Trial 2 | | | | | | | | | | | | | | | | | | |
| ECA | 5.23 a | 26.24 a | 0.32 a | 0.04 a | 5.54 b | 53.11 b | 4.44 a | 2.36 b | 3.34 a | 0.13 ab | 4.14 b | 1.89 b | 4.14 a | 1.85 b | 2.65 a | 0.74 b | 3.91 a | 0.80 a |
| ECC | 5.10 a | 27.76 a | T a | T a | 5.35 b | 49.65 b | 4.55 a | 2.36 b | 3.18 a | 0.02 b | 4.89 a | 4.34 a | 4.07 a | 2.15 b | 3.64 a | 1.02 b | 3.88 a | 1.58 a |
| CO | 4.89 a | 21.18 a | T a | T a | 5.07 a | 33.17 a | 4.70 a | 7.81 a | 3.41 a | 0.36 a | 4.50 ab | 1.54 b | 3.89 a | 3.18 a | 4.81 a | 7.73 a | 4.07 a | 3.18 a |

T = Trace

[1] Total area (Log of integrator units).

[2] Percentage of total peak areas.

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.

TABLE 5f. Chromatogram signatures for E. carotovora var. carotovora, E. carotovora var. atroseptica, wounded (CO) and non-wounded (CX) control treatments in two experimental trials.

| Treatment | Signatures[a] Trial 1 | Trial 2 |
|---|---|---|
| Areas[b] | | |
| ECA | C A H K F D G I E B | C A D H G K F E I : b |
| ECC | C H A K F I G D E B | C A G D H K F I E : b |
| CO | C A H K D G I F B : e | C A D G H K F I : be |
| CX | A C K H D I F G : be | |
| PTPA[c] | | |
| ECA | C A H K F D G I : eb | C A D H G K F E I : b |
| ECC | C H A K F I G D : eb | C A G D H K F I : eb |
| CO | C A H K D G I : fbe | C A D G H K F I : be |
| CX | A C K H D I F G : be | |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

TABLE 5g. Comparisons between signatures for E. carotovora var. carotovora, E. carotovora var. atroseptica, wounded (CO) and non-wounded (CX) control treatments in two experimental trials.

| | Characters Distinguishing Between Treatments[a] | | | | | |
|---|---|---|---|---|---|---|
| | ECC | | CO | | CX | |
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Areas[b] | | | | | | |
| ECA | H | G | EFHI | C | ACEFGH | (NA) |
| ECC | . | . | EHI | C | ADEFGH | (NA) |
| CX | . | . | AGH | (NA) | . | . |
| PTPA[c] | | | | | | |
| ECA | CFH | G | * | CDHI | CDI | (NA) |
| ECC | . | . | CFH | CDFHI | CDI | (NA) |
| CX | . | . | CDI | (NA) | . | . |

a) Characters which differ significantly between the treatments.

b) Log peak area.

c) Percentage of total peak areas.

* No characters significantly different.

(NA) Not applicable.

and compound H (CO > ECC).

In comparing the peak area values for the diseased (ECA and ECC) treatments with the non-wounded control (CX), all differentiations were again based on the greater total volatile production of the diseased treatments. The PTPA values for both the disease treatments (ECA and ECC) could be differentiated from the CX treatments on the basis of ethanol (ECA/ECC > CX) compound D (CX > ECA/ECC), and 3-hydroxy-2-butanone (CX > ECA/ECC).

The CO and CX treatments in Trial 1 could be differentiated on the basis of production levels for methanol (CO > CX), 1-butanol (CO > CX), and compound H (CO > CX). The PTPA values for ethanol (CO > CX), compound D (CX > CO), and 3-hydroxy-2-butanone (CX > CO) could be used to differentiate between the two treatments.

Differentiation between E. carotovora varieties. The test statistics for the covariant analyses of the regression equations and their slopes for the E. carotovora varieties (Table 5b) show that in Trial 1, the disease varieties could be differentiated on the basis of their daily volatile production regression line slopes ($P = 0.01$), but as a whole the regression equations were not significantly ($P = 0.05$) different. In Trial 2, neither the regression equations nor their slopes were significantly ($P = 0.05$) different for the volatile production regression of the two E. carotovora varieties.

In both experimental trials when the data were averaged over the entire test period the daily volatile outputs for the ECC and ECA treatments were not significantly ($P = 0.05$) different (Table 5c).

In both trials, all recorded chromatogram peaks were common to the volatile profiles of both E. carotovora varieties (REFERENCE TABLE C). The disease varieties did not produce a significantly ($P = 0.05$) different number of peaks/chromatogram in either experimental trial (Table 5d).

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the E. carotovora varieties are listed in Table 5e, and the corresponding chromatogram signatures are listed in Table 5f (see TABLE D for peak identification).

According to the test statistics in Table 5e, the metabolites which could be used to differentiate between the E. carotovora varieties are summarized in Table 5g. In Trial 1, compound H (ECC > ECA) could be used to differentiate between the disease varieties on the basis of mean compound production levels. In Trial 2, the disease varieties could be differentiated on the basis of the production levels of 1-butanol (ECC > ECA).

In Trial 1, the PTPA values for ethanol (ECA > ECC), the C-4 compounds (ECC > ECA), and compound H (ECC > ECA) were different for the E. carotovora varieties. In Trial 2, the disease varieties could be differentiated on the basis of the relative production levels of 1-butanol (ECC > ECA).

## Experiment 6: Volatile Profiles of Different Strains of *E. carotovora*

Disease summary. Contamination of control (CO) treatments was a persistent problem on both occasions that this experiment was conducted. In Trial 1, one of the three controls decayed due to infection by *E. carotovora* var. *atroseptica*. In Trial 2, three of the four control replicates showed some sign of decay due to *E. carotovora* var. *atroseptica* infection. Consequently, in Trial 2 the control treatments were omitted from the experimental analyses.

Disease development amongst the strains of *E. carotovora* was not uniform. In Trial 1, the ECC2 treatment did not develop as rapidly as the other infections. In Trial 2, the ECA2 and ECC3 treatments developed minimal visual symptomology relative to the other treatments. In both trials, there were considerable differences in the rate of disease development amongst the replicates for each treatment. As there were only two replicates/treatment in Trial 2, these replicate differences limited the validity of day to day analyses. Consequently, all day to day disease development statistics were limited to data from Trial 1.

*E. carotovora* bacteria were present in the bacterial seepage of all treatments which had showed extensive decay. Minimal *E. carotovora* populations were recovered from the infection sites in the treatments which had not decayed. Tests indicated that none of the variety specific infections had become contaminated by the opposing variety of *E. carotovora*.

In both trials, populations of secondary microflora were minimal in comparison to the *E. carotovora* populations. In Trial 1, *Staphylococcus* and *Clostridium* dominated the secondary microflora. In Trial 2, *Staphylococcus* and yeast were the predominant organisms.

## Volatile Production

Differentiation between strains within E. carotovora varieties. In all statistical tests of the data in Trial 2, the disease treatment X potato cultivar interactions were not statistically significant. In all subsequent analyses, the data was pooled accordingly.

In both experimental trials, there were clear-cut differences in the performance of the strains tested.

The mean daily total volatile production levels of the two strains of E. carotovora var. atroseptica (ECA1 and ECA2) are presented in Tables 6a and 6b. In Trial 1, the daily volatile output of ECA1 increased by a factor of 62X over the test period, while ECA2 increased by approximately 120 fold. Averaged over the entire incubation period, the total daily volatile output of ECA1 was approximately 50% greater than for ECA2, however, this difference was not statistically significant ($P = 0.05$) (Table 6c).

In Trial 2, the daily volatile outputs of ECA1 increased by 15X over the test period, but ECA2 did not show any appreciable increase in volatile output (Table 6b). Averaged over the test period, the total daily volatile output of ECA1 was approximately 20X greater than for ECA2, a difference significant at the 5% level (Table 6c).

In Trial 1, the daily volatile output of ECC1 increased by 119 fold over the test period, while ECC2 and ECC3 increased by factors of 15 and 106X, respectively (Table 6a). Averaged over the test period, the total daily volatile outputs for the three ECC strains were not significantly ($P = 0.05$) different (Table 6c).

In Trial 2, the daily volatile output of ECC1 increased by 17 fold over the test period, while ECC2 and ECC3 increased by factors of 14X and 12X, respectively (Table 6b). Averaged over the entire test period the total

TABLE 6a. Relative total peak areas[1)] for two strains of E. carotovora var. atroseptica (ECA) and three strains of E. carotovora var. carotovora (ECC) (Trial 1).

| Treatment | Incubation Period (Days) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | % | | | |
| ECA1 | 1.6[2)] | 9.9 | 49.2 | 100.0 |
| ECA2 | 0.6 | 0.3 | 20.7 | 71.2 |
| ECC1 | 0.3 | 6.7 | 32.6 | 35.7 |
| ECC2 | 0.4 | 0.7 | 2.6 | 6.1 |
| ECC3 | 0.4 | 2.9 | 14.5 | 42.3 |
| $\bar{X}$ ECA | 1.1 | 5.1 | 35.0 | 85.6 |
| $\bar{X}$ ECC | 0.4 | 3.4 | 16.6 | 28.0 |
| $\bar{X}$ Disease | 0.8 | 4.2 | 25.5 | 56.8 |
| Control | 0.4 | 0.5 | 3.4 | 5.3 |

[1)] Total peak area of ECA1 at 4 days = 100.

[2)] Value = peak area/peak area of ECA1 at 4 days X 100.

TABLE 6b. Relative total peak areas[1] for 'Russet Burbank' (R) and 'Norland' (N) tubers with three strains of E. carotovora var. carotovora (ECC), two strains of E. carotovora var. atroseptica (ECA), and control (CO) treatments (Trial 2).

| | Incubation Period (Days) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | % | | | |
| RECA1 | 4.6[2] | 3.0 | 26.4 | 36.6 |
| NECA1 | 0.3 | 0.9 | 28.9 | 37.9 |
| RECA2 | 0.8 | 0.2 | 0.3 | 0.7 |
| NECA2 | 1.0 | 0.3 | 0.9 | 0.5 |
| RECC1 | 1.2 | 17.6 | 16.4 | 21.0 |
| NECC1 | 1.3 | 17.3 | 26.8 | 19.7 |
| RECC2 | 9.3 | 56.0 | 99.2 | 100.0 |
| NECC2 | 2.8 | 42.9 | 59.8 | 67.8 |
| RECC3 | 0.6 | 0.8 | 0.6 | 8.9 |
| NECC3 | 0.3 | 0.1 | 0.5 | 0.7 |
| $\bar{X}$ ECA1 | 2.5 | 2.0 | 27.6 | 37.2 |
| $\bar{X}$ ECA2 | 0.9 | 0.2 | 0.6 | 0.6 |
| $\bar{X}$ ECC1 | 1.2 | 17.4 | 21.6 | 20.4 |
| $\bar{X}$ ECC2 | 6.0 | 49.4 | 79.5 | 83.9 |
| $\bar{X}$ ECC3 | 0.4 | 0.4 | 0.5 | 4.8 |
| $\bar{X}$ ECA | 1.7 | 1.1 | 14.1 | 18.9 |
| $\bar{X}$ ECC | 2.5 | 22.4 | 33.9 | 36.4 |
| $\bar{X}$ R | 3.3 | 15.5 | 28.5 | 33.4 |
| $\bar{X}$ N | 1.1 | 12.3 | 23.4 | 25.3 |

[1] Total peak area of RECC2 at 4 days = 100.

[2] Values = peak area/peak area of RECC2 at 4 days X 100.

TABLE 6c. Means of total of peak areas[1)] averaged over the incubation period for two strains of E. carotovora var. atroseptica (ECA) and three strains of E. carotovora var. carotovora (ECC).

| Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|
| Treatment | Mean | | Treatment | Mean | |
| ECA1 | 6.567* | a** | ECC2 | 6.633+ | a** |
| ECA2 | 6.391 | a | ECC1 | 6.060 | b |
| ECC1 | 6.307 | ab | ECA1 | 5.997 | b |
| ECC3 | 6.204 | b | ECC3 | 4.878 | c |
| ECC2 | 5.490 | bc | ECA2 | 4.761 | c |
| CO | 4.813 | c | | | |

1) Log of integrator unit values.

* Mean of three replicates.

+ Mean of four replicates.

** Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test of ordered means.

daily volatile outputs of all three ECC strains were significantly (P = 0.05) different (Table 6c).

In both trials, all major chromatogram peaks were common to the profiles of all E. carotovora strains tested (REFERENCE TABLE C). In both trials, ECA1 produced a significantly (P = 0.05) greater average number of peaks/chromatogram than ECA2 (Table 6d). In Trial 1, there were no significant (P = 0.05 differences in the average number of peaks/chromatogram for the E. carotovora var. carotovora strains tested. In Trial 2, ECC1 and ECC2 produced significantly (P = 0.05) greater numbers of peaks/chromatogram than ECC3 (Table 6d).

The total daily volatile outputs of the E. carotovora strains in Trial 1 were plotted as exponential functions in Figure 6a. The regression coefficients indicated that in all cases the total volatile outputs followed an exponential production pattern (P = 0.05 and 0.01) over the test period (Table 6e). The test statistics for the analysis of covariance for the regression equations derived to fit the total volatile production data are presented in Table 6f. ECA1 and ECA2 could not be differentiated on the basis of the regression slopes (P = 0.05) but the overall equations were significantly (P = 0.01) different. The regression equation for ECC2 could be differentiated from the equations for ECC1 and ECC3 on the basis of both slope and overall equation (P = 0.05). The regression equations for ECC1 and ECC3 were not significantly (P = 0.05) different.

The peak areas ($LOG_{10}$) and PTPA values, averaged over the entire incubation period for the major peaks in the chromatograms of the various strains of E. carotovora in the two experimental trials, are presented in Tables 6g and 6h. Table 6i contains the chromatogram signatures for the peak areas and PTPA values (see REFERENCE TABLE D for peak identification). The metabolites which, according to the test statistics in Tables 6g and 6h, could potentially be

TABLE 6d. Mean number of peaks/chromatogram averaged over the incubation period for two strains of E. carotovora var. atroseptica (ECA), and three strains of E. carotovora var. carotovora (ECC).

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Treatment | Mean | Treatment | Mean |
| ECA1 | 11.3* a** | ECC2 | 10.4+ a** |
| ECC3 | 9.3 ab | ECC1 | 8.9 ab |
| ECA2 | 8.2 bc | ECA1 | 8.5 b |
| ECC1 | 8.2 bc | ECC3 | 6.4 c |
| ECC2 | 7.1 bc | ECA2 | 6.0 c |
| CO | 5.8 c | | |

*Mean of three replicates.

+Mean of four replicates.

**Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test of ordered means.

FIGURE 6a. Daily total volatile production and corresponding best-fit regression lines for two strains of E. carotovora var. atroseptica (ECA), three strains of E. carotovora var. carotovora (ECC), and control (CO) treatments.

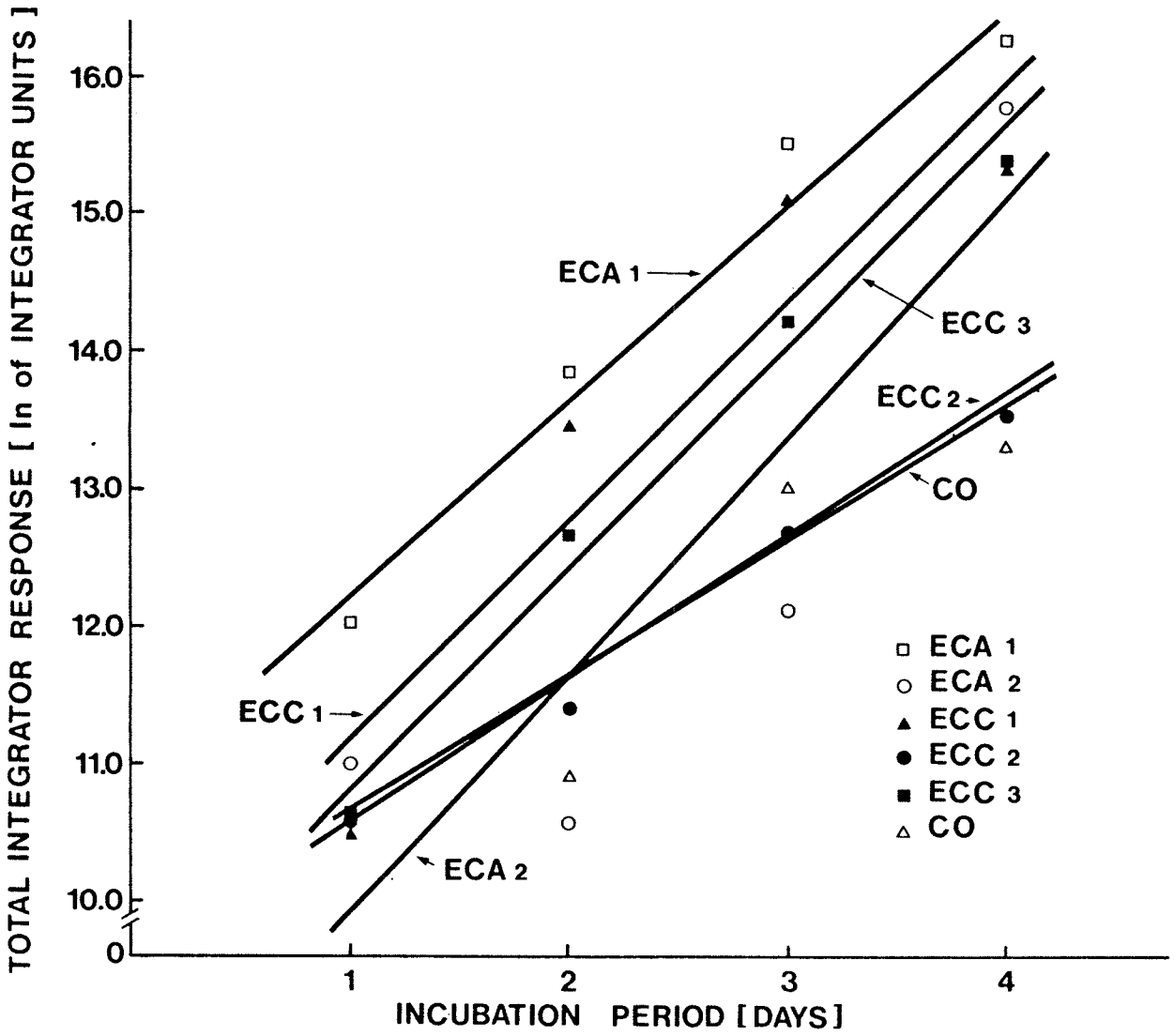

TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
16.0
15.0
14.0
13.0
12.0
11.0
10.0
0
1
2
3
4
INCUBATION PERIOD [ DAYS ]
ECA 1
ECC 3
ECC 2
CO
ECC 1
ECA 2
ECA 1
ECA 2
ECC 1
ECC 2
ECC 3
CO

TABLE 6e. Linear regression equations for the daily total integrator responses of three strains of E. carotovora var. carotovora (ECC) and two strains of E. carotovora var. atroseptica (ECA).

| Treatment | Equation | $r^2$ |
|---|---|---|
| ECA1 | $Y^{1)} = e^{11.91+1.40\overline{X}^{2)}}$ | $0.92^{**}$ |
| ECA2 | $Y = e^{8.29+1.65\overline{X}}$ | $0.71^{**}$ |
| ECC1 | $Y = e^{9.63+1.57\overline{X}}$ | $0.74^{**}$ |
| ECC2 | $Y = e^{9.52+0.97\overline{X}}$ | $0.62^{*}$ |
| ECC3 | $Y = e^{9.28+1.58\overline{X}}$ | $0.86^{**}$ |
| CO | $Y = e^{9.54+0.96\overline{X}}$ | $0.91^{**}$ |

Y = Total integrator response (integrator units).

$\overline{X}$ = Incubation period (days).

$^{**}$Significant at the 1% level.

$^{*}$Significant at the 5% level.

TABLE 6f. Analysis of covariance for the linear regression equations calculated for the daily total integrator responses[1)] of three strains of E. carotovora var. carotovora (ECC) and two strains of E. carotovora var. atroseptica (ECA).

| Pairwise comparison | F Test for slopes | F Test for similarity between lines |
|---|---|---|
| ECA1 vs ECA2 | $0.44^{N.S.}$ | $25.47^{**}$ |
| ECA1 vs ECC1 | $0.27^{N.S.}$ | $5.66^{*}$ |
| ECA1 vs ECC2 | $11.53^{**}$ | $35.58^{**}$ |
| ECA1 vs ECC3 | $20.60^{**}$ | $0.66^{N.S.}$ |
| ECA2 vs ECC1 | $0.60^{N.S.}$ | $5.54^{*}$ |
| ECA2 vs ECC2 | $6.61^{*}$ | $4.41^{*}$ |
| ECA2 vs ECC3 | $4.20^{N.S.}$ | $1.37^{N.S.}$ |
| ECC1 vs ECC2 | $7.22^{*}$ | $7.50^{*}$ |
| ECC1 vs ECC3 | $0.05^{N.S.}$ | $0.67^{N.S.}$ |
| ECC2 vs ECC3 | $10.66^{**}$ | $4.66^{*}$ |

$^{**}$Difference significant at the 1% level.

$^{*}$Difference significant at the 5% level.

N.S. Difference not significant at the 5% level.

1)Ln of integrator units.

TABLE 6g. Areas of chromatogram peaks averaged over the incubation period for two strains of E. carotovora var. atroseptica (ECA) and three strains of E. carotovora var. carotovora (ECC).

| | Methanol | | Ethanal | | Ethanol | | 2-Propanol | | RT = 7.1 min | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| ECA1 | 5.42 a* | 16.25 a | 1.56 b | T bc | 6.28 a | 50.37 a | 5.34 a | 10.50 b | 2.55 a | 0.75 a | 4.95 a | 3.00 a | 3.51 d | 0.62 c | 4.35 a | 2.63 a | 1.88 a | TR a | 4.72 a | 3.25 a |
| ECA2 | 3.98 a | 8.91 ab | 2.74 a | 3.17 bc | 5.39 a | 13.25 b | 5.44 a | 23.00 b | 2.72 a | 0.17 a | 4.49 ab | 0.83 a | 5.74 a | 22.33 a | 2.68 a | 0.17 ab | 1.26 a | TR a | 3.89 a | 7.17 a |
| ECC1 | 4.17 a | 3.63 b | 3.66 a | 4.83 abc | 4.41 ab | 4.04 b | 5.99 a | 54.33 a | 4.67 a | 2.45 a | 4.74 ab | 5.38 a | 4.98 ab | 11.58 b | 3.56 a | 0.04 b | 3.71 a | 1.08 a | 4.34 a | 2.54 a |
| ECC2 | 3.77 a | 6.58 b | 3.71 a | 9.00 ab | 2.44 b | 0.46 b | 4.56 a | 38.83 ab | 2.93 a | 2.33 a | 3.92 ab | 3.25 a | 3.88 cd | 9.96 b | 2.18 a | 0.33 ab | 1.48 a | 0.96 a | 3.91 a | 9.08 a |
| ECC3 | 2.93 a | 3.63 b | 3.91 a | 6.12 abc | 5.03 ab | 15.83 b | 5.22 a | 30.83 ab | 4.18 a | 5.04 a | 4.19 ab | 1.91 a | 4.72 bc | 6.08 c | 3.62 a | 0.25 ab | 1.96 a | 1.29 a | 3.11 a | 5.50 a |
| CO | 3.75 a | 5.33 b | 3.90 a | 14.00 a | 1.79 b | 0.83 b | 4.58 a | 36.50 ab | TR a | TR a | 3.08 b | 2.17 a | 3.61 cd | 6.17 bc | TR a | TR b | 3.93 a | 3.50 a | 3.86 a | 13.00 a |
| $\bar{X}$ ECA | 4.56 a | 11.85 a | 1.79 a | 1.90 b | 5.75 a | 28.10 a | 5.41 a | 18.00 b | 2.65 a | 0.40 a | 4.67 a | 1.70 a | 4.85 a | 13.65 a | 3.35 a | 1.15 a | 1.50 a | TR a | 4.30 a | 5.60 a |
| $\bar{X}$ ECC | 3.62 a | 4.61 b | 3.96 a | 6.65 a | 3.96 b | 6.78 b | 5.26 a | 41.33 a | 3.93 a | 3.28 a | 4.29 ab | 3.51 a | 4.53 ab | 9.20 b | 3.12 a | 0.21 a | 2.38 a | 1.11 a | 3.79 a | 5.70 a |
| CO | 3.75 a | 5.33 ab | 3.90 a | 14.00 a | 1.79 b | 0.83 b | 4.38 a | 36.50 ab | TR a | TR a | 3.08 b | 2.17 a | 3.61 b | 6.17 b | TR a | TR a | 3.93 a | 3.50 a | 3.86 a | 13.00 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.
T = Trace.
1)Total area (Log of integrator units).
2)Percentage of total peak areas.

TABLE 6h. Areas of chromatogram peaks averaged over the incubation period for 'Russet Burbank' (R) and 'Norland' (N) tubers inoculated with two strains of E. carotovora var. atroseptica (ECA) and three strains of E. carotovora var. carotovora (ECC).

| | Methanol | | Ethanal | | Ethanol | | 2-Propanol | | RT = 7.1 min | | C-4 pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| ECA1 | 3.19 a* | 9.83 a | 3.70 a | 5.94 c | 5.53 a | 22.67 b | 4.24 a | 3.78 a | T d | T b | 4.35 b | 5.72 b | 3.66 a | 2.72 b | 1.59 a | 0.44 a | 1.24 a | 0.11 a | 1.61 a | 0.22 c |
| ECA2 | T b | T b | 4.15 a | 32.88 a | 0.62 b | 0.17 c | 3.44 a | 8.67 a | 1.11 c | 0.46 b | 2.25 c | 0.75 b | 3.74 a | 14.00 a | 0.58 b | 0.25 a | 2.27 a | 1.33 a | 1.17 a | 10.96 a |
| ECC1 | 5.02 a | 7.71 ab | 2.84 a | 4.71 c | 5.91 a | 41.54 a | 4.03 a | 2.00 a | 3.04 b | 0.54 b | 4.34 b | 2.00 b | 4.17 a | 2.88 b | T b | T a | 3.06 a | 0.63 a | 2.17 a | 5.33 b |
| ECC2 | 3.88 a | 3.79 ab | 2.37 a | 1.54 c | 6.21 a | 37.92 ab | 4.20 a | 1.33 a | 5.07 a | 3.25 a | 5.96 a | 22.13 a | 4.08 a | 0.92 b | 3.29 a | 0.04 a | 3.30 a | 0.33 a | 4.25 a | T c |
| ECC3 | 3.42 a | 9.67 a | 3.81 a | 17.54 b | 1.82 b | 2.62 c | 3.55 a | 7.58 a | 1.48 c | 0.08 b | 2.54 c | 0.71 b | 3.87 a | 13.17 a | T b | T a | 1.57 a | 0.33 a | 2.22 a | 1.92 bc |
| $\bar{X}$ ECA | 1.37 b | 4.21 a | 3.96 a | 21.33 a | 2.73 b | 9.81 b | 3.79 a | 6.57 a | 0.63 b | 0.26 b | 3.15 b | 2.88 b | 3.71 a | 9.17 a | 1.01 a | 0.33 a | 1.83 a | 0.81 a | 2.98 a | 6.36 a |
| $\bar{X}$ ECC | 4.10 a | 7.06 a | 3.01 a | 7.93 b | 4.65 a | 27.36 a | 3.92 a | 3.64 a | 3.19 a | 1.29 a | 4.28 a | 8.28 a | 4.04 a | 5.65 a | 1.10 a | 0.01 a | 2.64 a | 0.43 a | 1.43 b | 2.42 b |
| R | 2.50 a | 4.00 b | 3.72 a | 13.61 a | 4.42 a | 23.83 a | 3.74 a | 3.50 a | 2.46 a | 0.57 a | 4.04 a | 7.19 a | 3.80 a | 6.67 a | 1.40 a | 0.01 a | 2.37 a | 0.44 a | 2.77 a | 4.22 a |
| N | 3.63 a | 7.81 a | 3.04 a | 12.20 a | 3.51 b | 18.25 a | 3.99 a | 5.82 a | 2.06 b | 1.22 a | 3.70 b | 5.48 a | 4.03 a | 7.20 a | 0.69 a | 0.25 a | 2.32 a | 0.68 a | 1.31 b | 3.55 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.
T = Trace
[1]Total area (Log of integrator units).
[2]Percentage of total peak areas.

TABLE 6i. Chromatogram signatures for two strains of E. carotovora var. atroseptica (ECA) and three strains of E. carotovora var. carotovora (ECC) in two experimental trials.

| Treatment | Signature[a] for area[b] | Signature for PTPA[c] |
|---|---|---|
| | Trial 1 | |
| ECA1 | C D A F G E K J B I H | C A D F K J G B E : h |
| ECA2 | G D C F A E J K B H I | D G C J A K B F E H : i |
| ECC1 | D G F C A I B E K J : h | D G F B C A K I E J : h |
| ECC2 | D K F G A B E C J I : h | D G K B A J F I E C : h |
| ECC3 | D C G F K B J E A I : h | D C B G K A F I T E : h |
| | Trial 2 | |
| ECA1 | C F I A G E B J K H D | C A F B G K I E H J D |
| ECA2 | B G E J F I K D C H : a | B G K J D E I F H C A |
| ECC1 | C A I F G J D B E K : h | C A B I G F K J D E : h |
| ECC2 | C F D I K G A J H B E | C F A D B K G I J D E H |
| ECC3 | G B A F E K I C J D : h | B G A K C E I F J D : h |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

used to differentiate between the strains of each E. carotovora variety in the two experimental trials are summarized in Table 6j. In each trial, the peak area values for a number of metabolites could be used to differentiate between the E. carotovora var. atroseptica strains. However, there was only a minimal correspondance between the results in the experimental trials. Often the results from the two trials were directly contradictory.

The PTPA values of ethanol (ECA1 > ECA2) and 1-butanol (ECA2 > ECA1) could be used to differentiate between the ECA strains in the two experimental trials. In comparing the three ECC strains, there were no compounds which were considered diagnostic either in terms of area or PTPA values in both treatments.

Differentiation between E. carotovora varieties and control treatments. The mean daily total volatile production values for the E. carotovora varieties were calculated by pooling and averaging the data for the respective strains of each variety. The mean daily total production levels for the experimental treatments are expressed as relative functions in Tables 6a and 6b. In Trial 1, the total volatile output of ECA increased by a factor of 78X over the incubation period, while ECC increased by 70 fold over its initial levels. Over the entire test period, the average volatile output of the ECA treatment was approximately 2.5X greater than for the ECC treatment. This difference was not significantly different at the 5% level (Table 6k).

The total volatile output of the control treatment in Trial 1 increased by 13 fold over the incubation period (Table 6a). The average volatile output of the controls was approximately 3% of the average for the diseased treatments (Table 6c). Both ECC and ECA had significantly ($P = 0.01$) greater average volatile production levels than the controls (Table 6k).

In Trial 2, the total daily volatile output of ECA increased by 11 fold

TABLE 6j. Comparisons within strains of signatures for E. carotovora var. atroseptica (ECA) and E. carotovora var. carotovora (ECC).

| | Characters Distinguishing Between Treatment[a] | | | | | |
|---|---|---|---|---|---|---|
| | ECA2 | | ECC1 | | ECC2 | |
| Areas[b] | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| ECA1 | BG | ACDFH | . | . | . | . |
| ECC1 | . | . | . | . | G | DFH |
| ECC3 | . | . | * | CDF | * | CDFH |
| PTPA[c] | | | | | | |
| ECA1 | CGJ | ABCGK | . | . | . | . |
| ECC1 | . | . | . | . | * | DFK |
| ECC3 | . | . | G | BCG | G | BCDFG |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

* No characters significantly different.

TABLE 6k. Pairwise comparisons of means of the total of peak areas[1)] for the disease treatments.

| Pairwise comparison | Trial # | Trmt | Mean total integrator response | t Value |
|---|---|---|---|---|
| ECA vs ECC | 1 | ECA | 6.437 | 1.81$^{N.S.}$ |
| | | ECC | 6.058 | |
| | 2 | ECA | 5.379 | 1.74$^{N.S.}$ |
| | | ECC | 6.037 | |
| ECA vs CO | 1 | ECA | 6.437 | 5.78$^{**}$ |
| | | CO | 4.813 | |
| | 2 | NA | | |
| ECC vs CO | 1 | ECC | 6.058 | 4.43$^{**}$ |
| | | CO | 4.813 | |
| | 2 | NA | | |

$^{**}$Significant at the 1% level.

N.S. Not significant at the 5% level.

1) Log of integrator unit values.

over the incubation period, while ECC increased by a factor of 14.5%. Over the entire test period the average volatile output of the ECC treatment was approximately 4.5% greater than the ECA treatments, however, this difference was not significant at the 5% level (Table 6k).

In both trials, all recorded chromatogram peaks were common to the volatile profiles of both E. carotovora varieties (REFERENCE TABLE C). A number of compounds common to the diseased profiles (i.e., 1-propanal/2-propanone and two unidentified compounds with RT of 7.1 and 14.1 min) were not detected in the volatile profiles of the control treatments.

In both trials, the ECA and ECC treatments could not be differentiated ($P = 0.05$) on the basis of the mean number of peaks/chromatogram (Table 61). In Trial 1, the control treatment produced significantly fewer peaks/chromatogram than the ECA ($P = 0.01$) and ECC treatments ($P = 0.05$) (Table 61).

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the ECC, ECA, and CO treatments are presented in Tables 6g and 6h, and the corresponding chromatogram signatures are presented in Table 6m (see REFERENCE TABLE D for peak identification). The metabolites which, according to the test statistics in Tables 6g and 6h, could potentially be used to differentiate between the treatments in the experimental trials are summarized in Table 6n. In each of the trials there were several compounds with peak area and PTPA values which, in theory, could be used to differentiate between the profiles for the E. carotovora varieties. Again, there was only minimal correspondence between the results in the two experimental trials. The results were again often completely contradictory. In both trials, the PTPA values for ethanal in the ECA profiles were significantly greater than in the ECC profiles.

In Trial 1, the control and ECA treatments could be differentiated on

TABLE 61. Pairwise comparisons of means of the number of peaks/chromatogram averaged over the incubation period for the disease treatments.

| Pairwise comparison | Test # | Trmt | Mean number peaks/chromatogram | t Value |
|---|---|---|---|---|
| ECA vs ECC | 1 | ECA | 9.78 | $2.1^{N.S.}$ |
| | | ECC | 8.19 | |
| | 2 | ECA | 7.25 | $1.59^{N.S.}$ |
| | | ECC | 8.57 | |
| ECA vs CO | 1 | ECA | 9.78 | $3.9^{**}$ |
| | | CO | 5.77 | |
| | 2 | NA | | |
| ECC vs CO | 1 | ECC | 8.19 | $2.9^{*}$ |
| | | CO | 5.77 | |
| | 2 | NA | | |

** Significant at the 1% level.

* Significant at the 5% level.

N.S. Not significant at the 5% level.

TABLE 6m. Chromatogram signatures for E. carotovora var. carotovora (ECC), E. carotovora var. atroseptica (ECA), and control treatments in two experimental trials.

| | Trial 1 | |
|---|---|---|
| | Signature[a] for areas[b] | Signature for PTPA[c] |
| ECA | C D G F A E K J B I H | C D G A J K F B E H : i |
| ECC | D G F C B A K E I J : h | D G C B K A F J I E : h |
| CO | D K I B A G F E C : hj | D B K G A I F C E : hj |
| | **Trial 2** | |
| ECA | B G F C E I J A K E H | B C G A F K I E J D H |
| ECC | C F A G I D B K J E H | C F B A G K I D E J H |
| CO | NA | NA |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak area.

TABLE 6n. Comparisons between signatures for E. carotovora var. atroseptica (ECA), E. carotovora var. carotovora (ECC), and control treatments in two experimental trials.

| | Characters Distinguishing Between Treatments[a] | | | |
|---|---|---|---|---|
| | ECC | | ECA | |
| | Areas[b] | PTPA[c] | Areas | PTPA |
| ECA | E | ABCG | · | · |
| CO | * | * | CFGJ | BCG |
| | Trial 2 | | | |
| ECA | ACDFK | BCDFK | · | · |
| CO | NA | NA | NA | NA |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

* No characters significantly different.

the basis of production levels of ethanol, the C-4 compounds, 1-butanol, and the unidentified compound J. In all cases, the ECA treatments had significantly greater compound production levels than the controls. The PTPA values for ethanal (CO > ECA), ethanol (ECA > CO), and 1-butanol (ECA > CO) were significantly different for the ECA and control treatments. The ECC and control treatments could not be differentiated on the basis of either the mean areas or PTPA values of any of the metabolites identified in this test.

Differentiation between potato cultivars. The mean daily total volatile output data for the two potato cultivars infected with E. carotovora bacteria was calculated by pooling and averaging the data from all disease treatments for each cultivar. The total volatile output of the infected 'Norland' (N) tubers increased by an average of 10% over the incubation period, while the 'Russet Burbank' (R) treatments increased by an average of 23 fold over their initial levels (Table 6b). Over the entire incubation period, the average volatile outputs of the potato cultivars were not significantly ($P = 0.05$) different (Appendix 6a).

All chromatogram peaks were common to the volatile profiles of both potato cultivars (REFERENCE TABLE C). The cultivar volatile profiles did not exhibit a significantly ($P = 0.05$) different mean number of peaks/chromatogram (Appendix 6b).

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the infected 'Norland' and 'Russet Burbank' tubers were presented in Table 6h; the corresponding chromatogram signatures were listed in Table 6o (see REFERENCE TABLE D for peak identification). The metabolites which, according to the test statistics in Table 6h, could potentially be used to differentiate between the volatile profiles of the potato cultivars are

TABLE 6o. Chromatogram signatures for 'Norland' and 'Russet Burbank' tubers infected with E. carotovora bacteria.

| | Signature[a] for areas[b] | Signatures for PTPA[c] |
|---|---|---|
| 'Norland' | G F A C I B J E D K H | C B A G F I K D E J H |
| 'Russet Burbank' | C F G B I K A D J E H | C B F G A K I D J E : h |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

summarized in Table 6p. The profiles of the potato cultivars could be differentiated on the basis of the average production levels of ethanol, compound D, the C-4 compounds, and compound K. In all cases, the differences were due to the greater volatile outputs of the 'Russet Burbank' treatment. Methanol (R>N) could be used to differentiate between the PTPA values of the potato cultivars.

TABLE 6p. Comparisons between signatures for 'Norland' and 'Russet Burbank' tubers infected with E. carotovora bacteria.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | 'Russet Burbank' | |
| | Areas[b] | PTPA[c] |
| 'Norland' | CDFK | A |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

Experiment 7: Effect of Incubation Temperature on Volatile Profiles of Diseased Potatoes

Disease summary. After 5 days at 22.5° C, the decay of the E. carotovora infected treatments (EMX) had progressed to a point where seepage prevented further volatile sampling of the incubation bags. The disease appeared to develop much more slowly at 9.8° C and there were only minimal indications of decay at 4.1° C after 7 days. At all three incubation temperatures, the control treatments (CO) remained 'healthy' throughout the test period.

E. carotovora bacteria were isolated from the bacterial seepage of the 22.5 and 9.8° C treatments at the end of the test period. At 4.1° C, the bacteria could only be isolated in the immediate vicinity of the inoculation points. Tests indicated that both varieties of E. carotovora were present in all diseased treatments at the end of the test period. Populations of secondary microorganisms were minimal in comparison to the E. carotovora populations. There were no appreciable differences in the diversity of microflora at the three incubation temperatures. Yeasts and Staphylococcus were identified as the predominant secondary microflora.

Volatile production. In all statistical analyses the disease treatment X incubation temperature interactions were non-significant ($P = 0.05$). (Appendix 7a and b). The data was pooled accordingly during the discussions of the main experimental variables.

The mean daily total volatile production values for the treatments have been plotted as exponential functions in Figure 7a, along with the corresponding best-fit regression lines. At the 22.5° C incubation temperature, the mean daily volatile production for the EMX treatment increased by 10X over the 5 day incubation period (Table 7a). At 9.8 and 4.1° C, the total volatile production of the EMX treatments increased by factors of 17X and 1.8X,

FIGURE 7a. Daily total volatile production and corresponding best-fit regression lines for a mixed E. carotovora (EMX) infection incubated at three temperatures.

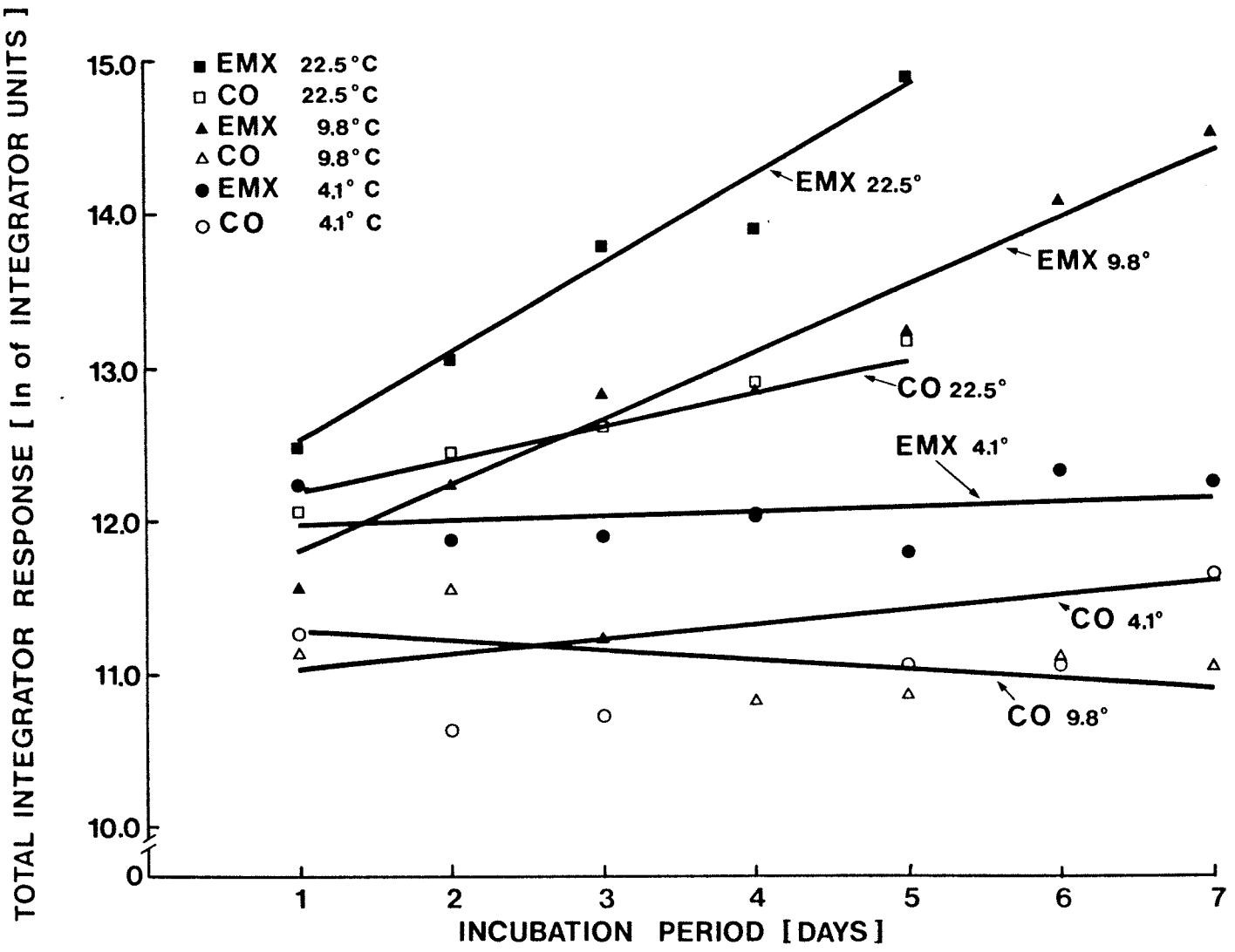

TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
15.0
14.0
13.0
12.0
11.0
10.0
0
■ EMX 22.5°C
□ CO 22.5°C
▲ EMX 9.8°C
△ CO 9.8°C
● EMX 4.1° C
○ CO 4.1° C
EMX 22.5°
EMX 9.8°
CO 22.5°
EMX 4.1°
CO 4.1°
CO 9.8°
1
2
3
4
5
6
7
INCUBATION PERIOD [DAYS]

TABLE 7a. Mean relative total peak areas[1] for a mixed E. carotovora infection incubated at three temperatures.

| | Incubation Period (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | % | | | | | | |
| Diseased 22.5° C | 9.4[2] | 17.0 | 36.1 | 39.5 | 100.0 | | |
| Control 22.5° C | 6.5 | 9.0 | 10.2 | 12.5 | 12.9 | | |
| Diseased 9.8° C | 4.4 | 8.2 | 13.3 | 12.9 | 19.4 | 52.7 | 74.8 |
| Control 9.8° C | 2.9 | 3.7 | 2.7 | 1.7 | 2.0 | 2.7 | 2.3 |
| Diseased 4.1° C | 7.4 | 5.2 | 5.4 | 6.1 | 4.7 | 8.9 | 7.1 |
| Control 4.1° C | 3.1 | 1.6 | 1.8 | 6.2 | 2.6 | 2.6 | 4.0 |
| $\bar{X}$ 22.5° C | 8.0 | 13.0 | 23.2 | 26.0 | 56.4 | | |
| $\bar{X}$ 9.8° C | 3.6 | 6.0 | 8.0 | 7.3 | 10.7 | 27.7 | 38.6 |
| $\bar{X}$ 4.1° C | 5.2 | 3.4 | 3.6 | 6.2 | 3.6 | 5.8 | 5.6 |
| $\bar{X}$ Diseased | 7.1 | 10.1 | 18.3 | 19.5 | 41.4 | | |
| $\bar{X}$ Control | 4.2 | 4.8 | 4.9 | 6.8 | 5.8 | | |

[1] Total peak area of diseased 22.5° C at 5 days = 100.

[2] Values = peak area/peak area of diseased 22.5° C at 5 days X 100.

respectively, over the 7 day incubation period (Table 7a).

At 22.5° C, the mean daily total volatile output of the controls increased by 2.3X over the test period (Table 7a). At the other two temperatures, volatile output by the controls remained relatively stable over the test period.

The regression coefficients for the exponential equations (Table 7b) indicated that the total daily volatile production of the EMX treatments at 22.5 and 9.8° C increased exponentially over the incubation period.

At each incubation temperature the regression equations and their slopes for the EMX treatments were significantly different (P = 0.05 or 0.01) (Table 7c). At 22.5 and 9.8° C, the diseased treatments could be differentiated from the corresponding control treatments on the basis of both the regression slopes (P = 0.05 or 0.01) and the general equations (P = 0.01). At 4.1° C, the slopes for the diseased and control regression equations were not significantly (P = 0.05) different.

The average total peak areas for the EMX treatments, pooled for the three incubation temperatures, were approximately 9X greater than those for the controls (Table 7d). This difference was significant at the 1% level. At 22.5° C, the average total peak areas, pooled for the disease and control treatments, were approximately 2.8X greater than at 9.8° C and approximately 4.4X greater than at 4.1° C (Table 7e). Only the difference between the 22.5 and 4.1° C output levels was significant at the 5% level.

The mean number of peaks/chromatogram for the EMX treatments, pooled for the three incubation temperatures, was significantly (P = 0.01) greater than for the controls (Table 7f). At 22.5° C, the mean number of peaks/chromatogram, pooled for the disease and control treatments, was significantly (P = 0.01) greater than at 9.8 and 4.1° C (Table 7g). The 9.8° C incubation

TABLE 7b. Linear regression equations for the daily total integrator responses for a mixed E. carotovora infection incubated at three temperatures.

| Treatment | Equation | $r^2$ |
|---|---|---|
| Diseased 22.5° C | $Y^{1)} = e^{11.93+0.56\bar{X}^{2)}}$ | $0.58^{**}$ |
| Control 22.5° C | $Y = e^{12.00+0.17\bar{X}}$ | $0.59^{N.S.}$ |
| Diseased 9.8° C | $Y = e^{11.26+0.45\bar{X}}$ | $0.65^{**}$ |
| Control 9.8° C | $Y = e^{11.35-0.06\bar{X}}$ | $0.10^{N.S.}$ |
| Diseased 4.1° C | $Y = e^{11.93+0.03\bar{X}}$ | $0.01^{N.S.}$ |
| Control 4.1° C | $Y = e^{10.96+0.07\bar{X}}$ | $0.06^{N.S.}$ |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

** Significant at the 1% level.

N.S. Not significant at the 5% level.

TABLE 7c. Analysis of covariance for the linear regression equations calculated for the daily total integrator responses[1)] of a mixed E. carotovora infection incubated at three temperatures.

| Pairwise comparison | F Test for slopes | F Test for similarity between lines |
|---|---|---|
| Diseased 22.5° C vs Control 22.5° C | 5.48* | 18.82** |
| Diseased 9.8° C vs Control 9.8° C | 19.31** | 43.00** |
| Diseased 4.1° C vs Control 4.1° C | 0.21N.S. | 14.21** |
| Diseased 22.5° C vs Diseased 9.8° C | 18.02** | 4.29* |
| Diseased 9.8° C vs Diseased 4.1° C | 20.33** | 20.81** |
| Diseased 22.5° C vs Diseased 4.1° C | 27.97** | 34.06** |

**Difference significant at the 1% level.

*Difference significant at the 5% level.

N.S. Difference not significant at the 5% level.

1) Ln of integrator units.

TABLE 7d. Pairwise comparisons of means of the total of peak areas[1)] averaged over three incubation temperatures for a mixed E. carotovora infection (EMX) and non-inoculated control (CO) treatments.

| Pairwise comparison | Mean total integrator response | t Value |
|---|---|---|
| EMX | 5.945 | 5.83** |
| vs | | |
| CO | 5.017 | |

**Values significantly different at the 1% level.

1)Log of sum of all individual peak areas.

TABLE 7e. Pairwise comparisons of means of the total peak areas[1)] for three incubation temperatures.

| Pairwise comparison | Mean total integrator response | t Value |
|---|---|---|
| 22.5° C | 5.971 | 2.18$^{N.S.}$ |
| vs | | |
| 9.8° C | 5.574 | |
| 22.5° C | 5.971 | 3.39** |
| vs | | |
| 4.1° C | 5.326 | |
| 9.8° C | 5.574 | 1.30$^{N.S.}$ |
| vs | | |
| 4.1° C | 5.326 | |

** Values significantly different at the 1% level.

N.S. Values not significantly different at the 5% level.

1) Log of sum of all individual peaks.

TABLE 7f. Pairwise comparison of mean number of peaks/ chromatogram for a mixed E. carotovora infection (EMX) averaged over three incubation temperatures.

| Pairwise comparison | Mean number of peaks/ chromatogram | t Value |
|---|---|---|
| EMX | 11.91 | |
| vs | | 9.7** |
| Control | 9.83 | |

** Values significantly different at the 1% level.

TABLE 7g. Pairwise comparisons of means of the average number of peaks/chromatogram for the three incubation temperatures.

| Pairwise comparison | Mean number of peaks chromatogram | t Value |
|---|---|---|
| 22.5° C | 8.77 | |
| vs | | 3.32** |
| 9.8° C | 7.93 | |
| 22.5° C | 8.77 | |
| vs | | 7.63** |
| 4.1° C | 6.76 | |
| 9.8° C | 7.93 | |
| vs | | 4.44** |
| 4.1° C | 6.76 | |

** Values significantly different at the 1% level.

temperature also produced a significantly (P = 0.01) greater mean number of peaks/chromatogram than at 4.1° C.

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the experimental treatments are presented in Table 7h; the corresponding chromatogram signatures are presented in Tables i, j, and k (see REFERENCE TABLE D for peak identification).

The metabolites which, according to the test statistics in Table 7h, could potentially be used to differentiate between the volatile profiles of the experimental treatments are summarized in Tables 71, m, and n. The EMX 22.5° C treatment could be differentiated from the corresponding control on the basis of production levels of methanol (EMX > CO). No significantly different PTPA values were recorded. The EMX 9.8° C treatment could be differentiated from the corresponding control on the basis of production levels of methanol (EMX > CO) and the PTPA values for methanol (EMX > CO) and the compound K (CO > EMX). The EMX 4.1° C treatment could be differentiated from the corresponding control profile on the basis of production levels of methanol (EMX > CO) and ethanol (EMX > CO) and the PTPA values for ethanol (EMX > CO).

The EMX 22.5° C treatment could be differentiated from the EMX 9.8° C treatment on the basis of the PTPA values for methanol (EMX 9.8° C > EMX 22.5° C), ethanal (EMX 22.5° C > EMX 9.5° C), and 3-hydroxy-2-butanone (EMX 9.8° C > EMX 22.5° C). The EMX 22.5° C and EMX 4.1° C treatments had significantly different production values for ethanol (EMX 22.5 > EMX 4.1) and significantly different PTPA values for methanol (EMX 4.1 > EMX 22.5), ethanal (EMX 22.5 > EMX 4.1), and compound K (EMX 4.1 > EMX 22.5). The EMX 9.8° C treatment could be differentiated from the EMX 4.1° C treatment on the basis of the average production levels for methanol (EMX 9.8 > EMX 4.1) and on the basis of the PTPA values for methanol (EMX 4.1 > EMX 9.8), 3-hydroxy-2-butanone (EMX 9.8 > EMX 4.1), and compound K (EMX 4.1 > EMX 9.8).

TABLE 7h. Areas of chromatogram peaks averaged over the incubation period for a mixed E. carotovora infection (EMX) held at three incubation temperatures.

| | Methanol | | Ethanal | | Ethanol | | 2-Propanol | | RT = 7.1 min | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| EMX 22.5° C | 4.27 ab* | 1.36 d | 4.38 a | 2.77 a | 4.78 a | 5.14 a | 3.82 a | 0.68 a | 3.37 a | 0.58 a | 4.73 a | 9.33 a | 4.32 a | 1.66 a | 0.99 a | 0.02 a | 2.96 a | 1.45 c | 1.69 a | 0.56 c |
| Control 22.5° C | T c | T d | 3.78 a | 2.38 ab | 2.80 ab | 0.31 a | 2.45 a | 0.19 a | T a | T a | 3.63 a | 1.44 a | 2.41 a | 1.19 a | T a | T a | 3.39 a | 0.88 c | 2.35 a | 1.13 c |
| EMX 9.8° C | 5.04 a | 18.28 b | 1.42 ab | 0.22 c | 4.65 a | 9.16 a | 3.25 a | 1.52 a | 2.40 a | 0.59 a | 4.49 a | 6.70 a | 3.86 a | 2.39 a | 1.45 a | 0.75 a | 4.67 a | 7.27 b | 3.51 a | 2.25 c |
| Control 9.8° C | 3.91 b | 10.56 c | T b | T c | 2.74 ab | 0.75 a | 2.78 a | 1.13 a | 2.65 a | 0.81 a | 3.28 a | 3.31 a | 3.47 a | 3.81 a | T a | T a | 3.65 a | 8.00 b | 4.09 a | 17.19 a |
| EMX 4.1° C | 4.84 a | 31.52 a | 2.74 ab | 0.81 bc | 2.34 b | 1.10 a | 3.49 a | 1.56 a | 2.05 a | 0.83 a | 2.44 a | 2.44 a | 2.41 a | 3.23 a | 1.27 a | 1.06 a | 4.15 a | 2.88 c | 3.75 a | 5.28 b |
| Control 4.1° C | 4.01 b | 24.75 ab | 3.40 a | 2.25 ab | T c | T a | 2.84 a | 2.38 a | T a | T a | 2.69 a | 1.13 a | 3.50 a | 4.63 a | T a | T a | 2.91 a | 14.50 a | 2.86 a | 6.75 b |
| $\bar{X}$ 22.5° C | 4.14 a | 8.73 b | 3.01 a | 1.59 a | 4.50 a | 6.39 a | 3.41 a | 0.99 a | 2.56 a | 0.52 a | 4.50 a | 7.28 a | 3.90 a | 1.93 a | 1.08 a | 0.34 a | 3.77 a | 3.97 a | 2.58 a | 1.38 c |
| $\bar{X}$ 9.8° C | 3.91 a | 10.56 b | 0.41 a | 0.16 a | 2.74 ab | 0.75 a | 2.78 a | 1.13 a | 2.65 a | 0.81 a | 3.29 a | 3.31 a | 3.47 a | 3.81 a | 0.91 a | 0.11 a | 3.65 a | 8.00 a | 4.09 a | 17.18 a |
| $\bar{X}$ 4.1° C | 4.63 a | 29.83 a | 2.91 a | 1.17 a | 1.76 b | 0.83 a | 3.33 a | 1.77 a | 1.54 a | 0.63 a | 2.50 a | 2.11 a | 2.68 a | 3.58 a | 0.94 a | 0.80 a | 3.84 a | 5.78 a | 3.53 a | 5.98 b |
| $\bar{X}$ EMX | 4.71 a | 15.74 a | 2.86 a | 1.31 a | 4.07 a | 5.50 a | 3.52 a | 1.22 a | 2.66 a | 0.65 a | 4.02 a | 6.49 a | 3.63 a | 2.35 a | 1.23 a | 0.56 a | 3.90 a | 3.95 a | 2.92 a | 2.59 b |
| $\bar{X}$ Control | 2.64 b | 11.77 a | 2.39 a | 1.54 a | 1.85 b | 0.35 a | 2.69 a | 1.23 a | 0.88 a | 0.27 a | 3.20 a | 1.96 a | 3.13 a | 3.20 a | T a | T a | 3.32 a | 7.79 a | 3.10 a | 8.35 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.
T = Trace.

[1]Total area (Log of integrator units).

[2]Percentage of total peak areas.

TABLE 7i. Chromatogram signatures between a mixed *E. carotovora* infection (EMX) and non-inoculated controls (CO) at three incubation temperatures.

| Treatment | Signature[a] for areas[b] | Signatures for PTPA[c] |
|---|---|---|
| EMX 22.5° C | C F B G A D I E J K H | F C B D G I A K J E H |
| CO 22.5° C | B K F I E C G : adhj | B K F G I E C : adhj |
| EMX 9.8° C | A I C F G K J E D H B | A C J I F K G H E D B |
| CO 9.8° C | K A I G F E C D : bhj | K A I G F E D C : bhj |
| EMX 4.1° C | A I K E B F G C D H : j | A K G I F E C H D B : j |
| CO 4.1° C | K A G B E I F : cdhj | A I K G B E F : cdhj |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 7j. Chromatogram signatures of volatile profiles recorded at three incubation temperatures.

| Treatment | Signature[a] for areas[b] | Signatures for PTPA[c] |
|---|---|---|
| 4.1° C | A I K E B G F C D H : j | A K I G F E B C H D : j |
| 9.8° C | A I C F G K E J D H B | A C I F J K G E D H B |
| 22.5° C | F C B G A I D E K J H | F C B D G K I A J E H |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

TABLE 7k. Chromatogram signatures for a mixed E. carotovora infection (EMX) and control treatments.

| Treatment | Signature[a] for areas[b] | Signatures for PTPA[c] |
|---|---|---|
| EMX | A C F I G E K B D J H | A F C I J K G B D E H |
| Control | I F G K E A B C D : hj | A K I G F B E C D : hj |

[a] Peaks significantly (P = 0.05) different from one another at Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak area.

TABLE 71. Comparison between chromatogram signatures for a mixed *E. carotovora* infection (EMX) and non-inoculated controls (CO) at three incubation temperatures.

| | Characters Distinguishing Between Treatments[a] | | | | | |
|---|---|---|---|---|---|---|
| | EMX 22.5° C | | EMX 9.8° C | | EMX 4.1° C | |
| | Area[b] | PTPA[c] | Area | PTPA | Area | PTPA |
| CO 22.5° C | A | * | . | . | . | . |
| EMX 9.8° C | * | ABI | . | . | . | . |
| CO 9.8° C | . | . | A | AK | . | . |
| EMX 4.1° C | C | ABK | C | AIK | . | . |
| CO 4.1° C | . | . | . | . | AC | I |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

* No characters significantly different.

TABLE 7m. Comparison between chromatogram signatures for the volatile profiles recorded at three incubation temperatures.

| | Characters Distinguishing Between Treatments[a] | | | |
|---|---|---|---|---|
| | 9.8° C | | 22.5° C | |
| | Area[b] | PTPA[c] | Area | PTPA |
| 4.1° C | J | AK | C | AK |
| 9.8° C | . | . | * | K |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

* No characters significantly different.

TABLE 7n. Comparison between chromatogram signatures for a mixed E. carotovora infection and control treatments.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | E. carotovora Infected | |
| | Area[b] | PTPA[c] |
| Control | AC | K |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

When the experimental data were pooled according to the three incubation temperatures the volatile profiles for the experimental trial incubated at 4.1° C could be differentiated from the profiles at 9.8° C on the basis of the production levels of compound J (9.8 > 4.1) and on the basis of the PTPA values for methanol (9.8 > 4.1), and compound K (9.8 > 4.1). The 22.1 and 4.1° C treatments could be differentiated on the basis of the production levels for ethanol (22.5 > 4.1) and the PTPA values for methanol (22.1 > 4.1), and compound K (4.1 > 22.5). The 9.8 and 22.5° C treatments could only be differentiated on the basis of the PTPA values for compound K (9.8 > 22.5).

When the experimental data was pooled according to the disease treatments (EMX vs CO) the profiles for the two treatments could be differentiated on the basis of average production levels of methanol (EMX > CO) and ethanol (EMX > CO). The PTPA values for compound K (CO > EMX) were also significantly different in the volatile profiles of the disease and control treatments.

Experiment 8: Effect of Incubation Temperature on Volatile Profiles of Potatoes Infected with E. carotovora var. carotovora and E. carotovora var. atroseptica

Disease summary. In both experimental trials, disease development amongst the treatment replicates was relatively uniform. The soft-rot symptomology appeared to develop more rapidly at the higher (22.8° C) incubation temperature. All control treatments remained sound throughout the test periods.

E. carotovora bacteria were recovered from the bacterial seepage of all inoculated treatments. Tests indicated that none of the variety specific infections had become contaminated by the opposing E. carotovora variety. There were no immediately appreciable differences between the populations of the two E. carotovora varieties at each incubation temperature.

In both trials, populations of secondary microflora were minimal in comparison to the E. carotovora populations. There were no significant differences in the size or nature of the secondary microflora communities at the two incubation temperatures. In both trials, Staphylococcus, E. coli, and yeasts were the predominant secondary organisms.

Volatile production. The total daily volatile output data for the experimental treatments in Trial 1 have been plotted as a natural log function in Figure 8a along with the corresponding best-fit regression lines. The data in Trial 2 showed similar volatile production patterns. The total daily volatile output data for the ECC 22.8° C ($P = 0.01$), CO 22.8° C ($P = 0.05$), ECA 9.8° C ($P = 0.05$), and ECC 9.8° C ($P = 0.01$) treatments followed an exponential pattern of increase over the test period (Table 8a).

The analysis of variance statistics for the total volatile outputs of the various experimental treatments, averaged over the entire test period,

FIGURE 8a. Daily total volatile production and corresponding best-fit regression lines for the E. carotovora varieties (ECC and ECA) incubated at two temperatures (Trial 1).

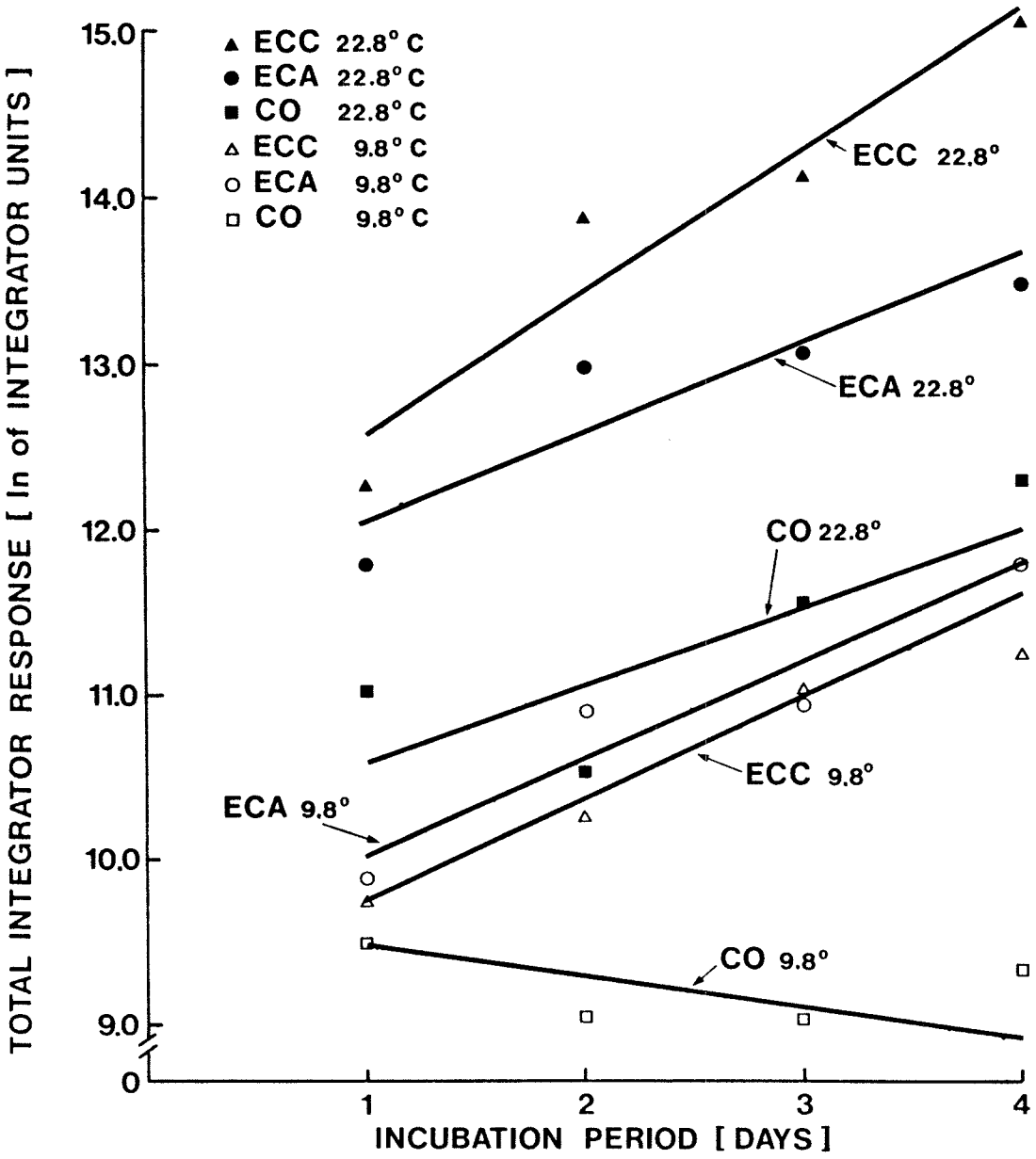

▲ ECC 22.8° C
● ECA 22.8° C
■ CO 22.8° C
△ ECC 9.8° C
○ ECA 9.8° C
□ CO 9.8° C
ECC 22.8°
ECA 22.8°
CO 22.8°
ECC 9.8°
ECA 9.8°
CO 9.8°
TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
15.0
14.0
13.0
12.0
11.0
10.0
9.0
0
1
2
3
4
INCUBATION PERIOD [ DAYS ]

TABLE 8a. Linear regression equations for the daily total integrator responses of the E. carotovora varieties (ECC and ECA) and non-inoculated control (CO) treatments incubated at two different temperatures (Trial 1).

| Treatment | Equation | $r^2$ |
|---|---|---|
| ECA 22.8° C | $Y^{1)} = e^{11.53+0.52\overline{X}^{2)}}$ | $0.46^{N.S.}$ |
| ECC 22.8° C | $Y = e^{11.74+0.85\overline{X}}$ | $0.75^{**}$ |
| CO 22.8° C | $Y = e^{10.16+0.46\overline{X}}$ | $0.60^{*}$ |
| ECA 9.8° C | $Y = e^{9.49+0.56\overline{X}}$ | $0.55^{*}$ |
| ECC 9.8° C | $Y = e^{9.20+0.57\overline{X}}$ | $0.83^{**}$ |
| CO | $Y = e^{9.21-0.21\overline{X}}$ | $0.04^{N.S.}$ |

1) $Y$ = Total integrator response (integrator units).

2) $\overline{X}$ = Incubation period (days).

TABLE 8b. Analysis of variance for means of total integrator responses[1)] averaged over the incubation period for E. carotovora var. atroseptica or E. carotovora var. carotovora inoculated potatoes held at two incubation temperatures.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Trial 1 | | | | |
| Disease (D) | 2 | 1.867 | 0.934 | 18.74** |
| Temperature (T) | 1 | 2.433 | 2.433 | 48.86** |
| DXT | 2 | 0.538 | 0.269 | 5.40* |
| Error | 12 | 0.598 | 0.050 | |
| Total | 17 | 5.436 | | |
| Trial 2 | | | | |
| Disease (D) | 2 | 1.858 | 0.929 | 66.36** |
| Temperature (T) | 1 | 5.619 | 5.619 | 401.36** |
| DXT | 2 | 0.402 | 0.201 | 14.36** |
| Error | 12 | 0.162 | 0.014 | |
| Total | 17 | 8.041 | | |

**Significant at the 1% level.

*Significant at the 5% level.

1)Log of sum of all individual peak areas (integrator units).

are presented in Table 8b. In both trials, the disease (D) treatment (P = 0.01) incubation temperature (T), and DXT interaction (P = 0.05 or 0.01) effects were found to be statistically significant. The average daily volatile outputs of the ECA and ECC treatments were not significantly different (P = 0.05). However, both disease treatments had a significantly (P = 0.05) greater average total volatile output than the controls ($\overline{X}$ = 50X controls) (Table 8c). In both trials, the 22.5° C treatments produced significantly greater average daily volatile outputs than the 9.8° C treatments (P = 0.05) (Table 8c).

In both trials, the significance of the DXT interaction stemmed from the fact that at 22.5° C the ECC treatment consistently produced greater total volatile outputs than the ECA treatment, whereas at 9.8° C the ECA treatment produced the greater average volatile outputs (Table 8c).

Table 8d contains the test statistics for the covariant analysis of the regression equations derived for the experimental treatments. At the 22.8° C incubation temperature, the regression equations for the total daily volatile production levels of the experimental treatments (ECC, ECA, CO) were significantly different (P = 0.01) but the slopes of the lines were not different (P = 0.05). At 9.8° C, the ECA and ECC regression equations were not significantly different (P = 0.05), but both disease treatments could be differentiated from the CO volatile production response.

When each E. carotovora variety was compared at the two incubation temperatures, the slopes for the regression lines were not significantly (P = 0.05) different, however, in all cases the overall equations at the two incubation temperatures were significantly (P = 0.01) different.

In both trials, all recorded chromatogram peaks were common to the volatile profiles of the diseased treatments (REFERENCE TABLE C). The average

TABLE 8c. Means of total integrator responses[1)] averaged over the incubation period for E. carotovora var. carotovora (ECC) or E. carotovora var. atroseptica (ECA) infected potatoes incubated at two temperatures.

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Treatment | Means | Treatment | Means |
| ECC 22.5° C | 6.247 a* | ECC 22.5° C | 6.181 a |
| ECA 22.5° C | 5.741 ab | ECA 22.5° C | 5.685 b |
| ECA 9.8° C | 5.219 bc | CO 22.5° C | 5.156 c |
| CO 22.5° C | 5.103 c | ECA 9.8° C | 4.841 cd |
| ECC 9.8° C | 4.982 c | ECC 9.8° C | 4.647 d |
| CO 9.8° C | 4.684 c | CO 9.8° C | 4.182 e |
| $\bar{X}$ 22.5° C | 5.697 a | $\bar{X}$ 22.5° C | 5.674 a |
| $\bar{X}$ 9.8° C | 4.962 b | $\bar{X}$ 9.8° C | 4.557 b |
| $\bar{X}$ ECC | 5.615 a | $\bar{X}$ ECC | 5.414 a |
| $\bar{X}$ ECA | 5.480 a | $\bar{X}$ ECA | 5.263 a |
| $\bar{X}$ CO | 4.893 b | $\bar{X}$ CO | 4.669 b |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test of ordered means.

1)Log of sum of all individual peak areas (integrator units).

TABLE 8d. Analysis of covariance for regression equations calculated for the daily total integrator responses[1)] for the E. carotovora varieties (ECC and ECA) incubated at two different temperatures.

| Pairwise comparison | F Test for slope | F Test for similarity between lines |
|---|---|---|
| ECA 22.8° C vs ECC 22.8° C | 1.43 N.S. | 11.37** |
| ECA 22.8° C vs CO 22.8° C | 0.01 N.S. | 16.17** |
| ECC 22.8° C vs CO 22.8° C | 1.29 N.S. | 43.81** |
| ECA 9.8° C vs ECC 9.8° C | 0.01 N.S. | 1.86 N.S. |
| ECA 9.8° C vs CO 9.8° C | 4.00* | 11.99** |
| ECC 9.8° C vs CO 9.8° C | 14.00** | 15.45** |
| ECA 22.8° C vs ECA 9.8° C | 0.01 N.S. | 53.33** |
| ECC 22.8° C vs ECC 9.8° C | 2.14 N.S. | 222.61** |
| CO 22.8° C vs CO 9.8° C | 2.76 N.S. | 18.83** |

** Difference significant at the 1% level.

N.S. Difference not significant at the 5% level.

1) Ln of integrator values.

TABLE 8e. Analysis of variance for mean number of peaks/chromatogram averaged over the incubation period for E. carotovora var. carotovora or E. carotovora var. atroseptica infected potatoes incubated at two temperatures.

| Source of variation | | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|---|
| Trial 1 | | | | | |
| Disease | (D) | 2 | 14.51 | 7.25 | 13.61** |
| Temperature | (T) | 1 | 34.31 | 34.31 | 64.37** |
| DXT | | 2 | 1.33 | 0.67 | 1.21N.S. |
| Error | | 12 | 6.40 | 0.55 | |
| Total | | 17 | 56.42 | | |
| Trial 2 | | | | | |
| Disease | (D) | 2 | 26.40 | 13.20 | 52.8 ** |
| Temperature | (T) | 1 | 10.73 | 10.73 | 42.9 ** |
| DXT | | 2 | 2.22 | 1.11 | 4.4 * |
| Error | | 12 | 3.01 | 0.25 | |
| Total | | 17 | 42.36 | | |

**Significant at the 1% level.

*Significant at the 5% level.

N.S. Not significant at the 5% level.

TABLE 8f. Mean number of peaks/chromatogram averaged over the incubation period for E. carotovora var. carotovora (ECC) or E. carotovora var. atroseptica (ECA) infected potatoes incubated at two temperatures.

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Treatment | Means | Treatment | Means |
| ECA 22.5° C | 10.4 a* | ECC 22.5° C | 7.8 a |
| ECC 22.5° C | 9.3 ab | ECA 22.5° C | 7.3 ab |
| CO 22.5° C | 7.6 bc | ECA 9.8° C | 6.2 bc |
| ECA 9.8° C | 7.0 cd | ECC 9.8° C | 5.6 cd |
| ECC 9.8° C | 6.6 cd | CO 22.5° C | 4.8 d |
| CO 9.8° C | 5.5 d | CO 9.8° C | 3.4 e |
| $\bar{X}$ 22.5° C | 9.1 a | $\bar{X}$ 22.5° C | 6.6 a |
| $\bar{X}$ 9.8° C | 6.4 b | $\bar{X}$ 9.8° C | 5.1 b |
| $\bar{X}$ ECA | 8.7 a | $\bar{X}$ ECA | 6.7 a |
| $\bar{X}$ ECC | 8.0 a | $\bar{X}$ ECC | 6.7 a |
| $\bar{X}$ CO | 6.6 b | $\bar{X}$ CO | 4.1 b |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test of ordered means.

number of peaks/chromatogram for the ECC and ECA treatments were not significantly different in either of the trials (P = 0.05) (Tables 8e and 8f). Both disease treatments had a significantly (P = 0.05) greater mean number of peaks/chromatogram than the controls (Table 8e). The 22.8° C treatments also had a significantly (P = 0.05) greater average number of peaks/chromatogram than the 9.8° C treatment. In Trial 1, the DXT interaction effect was not significant (P = 0.05) in the analysis of variance of the mean number of peaks/chromatogram (Table 8e). In Trial 2, the DXT effect was significant (P = 0.05) as at 22.8° C ECC > ECA, while at 9.8° C ECA > ECC.

The mean peak areas ($LOG_{10}$) and PTPA values for the major peaks in the chromatograms of the ECC, ECA, and CO treatments are listed in Table 8g; the corresponding chromatogram signatures are presented in Table 8h (see REFERENCE TABLE D for peak identification). The metabolites which, according to the test statistics in Table 8g, could potentially be used to differentiate between the ECC, ECA, and CO treatments are summarized in Table 8i. In both trials, the peak areas and PTPA values for a number of components in the volatile profiles of the disease treatments were found to be significantly different. However, there was very little correspondence between the diagnostic characteristics established in the two trials. In the peak area comparisons, in the majority of cases differentiation between the disease treatments and the controls was due to greater outputs by the diseased treatments.

The peak areas ($LOG_{10}$) and PTPA values, averaged over the incubation period, for the major peaks in the chromatograms of the incubation temperature treatments are listed in Table 8g. Table 8j contains the chromatogram signatures for the mean volatile output and PTPA values. The metabolites, which according to the test statistics in Table 8g could be used to differentiate between the volatile profiles at the two incubation temperatures, are summarized in Table 8k. In both trials, the two incubation temperatures produced

TABLE 8g. Areas of chromatogram peaks averaged over the incubation period for the disease treatments (ECA, ECC and CO) and the two incubation temperatures.

| | Methanol | | Ethanal | | Ethanol | | 2-Propanol | | RT = 7.1 min | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| Trial 1 | | | | | | | | | | | | | | | | | | | | |
| ECA | 4.16 a* | 18.00 a | 1.56 a | 3.24 a | 3.79 a | 8.32 a | 1.85 b | 2.16 a | 2.09 a | 0.60 a | 3.17 a | 2.72 b | 3.59 a | 9.16 a | T b | T a | 2.18 b | 2.36 a | 4.26 a | 19.88 a |
| ECC | 3.88 a | 12.00 a | 2.17 a | 5.47 a | 3.00 a | 8.83 a | 3.66 a | 3.83 a | 0.51 a | 0.10 a | 3.93 a | 13.53 a | 3.47 a | 7.57 ab | 1.34 a | 0.17 a | 4.13 a | 9.50 a | 2.55 a | 11.07 a |
| CO | 4.06 a | 17.20 a | 0.52 a | 0.20 a | 1.03 b | 0.40 a | 1.08 b | T a | 0.93 a | T a | 1.82 a | 2.20 b | 3.22 a | 2.30 b | T b | T a | 2.00 b | 3.60 a | 1.58 a | 2.50 a |
| 22.8° C | 4.95 a | 19.30 a | 0.48 b | 0.03 b | 4.14 a | 11.73 a | 3.81 a | 2.30 a | 0.53 a | 0.03 a | 4.54 a | 12.73 a | 3.33 a | 1.03 b | 1.34 a | 0.17 a | 4.22 a | 5.67 a | 3.47 a | 5.90 a |
| 9.8° C | 3.22 a | 11.51 a | 2.70 a | 7.03 a | 2.02 b | 3.57 b | 1.51 b | 2.85 a | 1.74 a | 0.49 a | 2.27 b | 3.26 b | 3.60 a | 12.80 a | T b | T a | 2.06 b | 6.00 a | 2.70 a | 19.34 a |
| Trial 2 | | | | | | | | | | | | | | | | | | | | |
| ECA | 4.40 a | 21.52 a | 0.46 b | 0.72 a | 3.70 a | 10.52 a | 2.59 a | 0.88 a | 2.82 a | 1.72 a | 2.33 a | 3.16 a | 4.22 a | 13.12 a | 3.26 a | 1.72 a | 3.82 a | 5.68 a | 2.68 a | 7.68 a |
| ECC | 2.97 a | 5.00 b | 2.79 ab | 5.48 a | 2.43 a | 12.08 a | 1.16 a | 2.76 a | 2.46 a | 9.08 a | 1.53 a | 0.96 a | 3.79 a | 20.80 a | 2.50 a | 3.16 a | 2.89 a | 4.92 a | 2.89 a | 8.64 a |
| CO | T b | T b | 3.70 a | 9.80 a | 2.26 a | 3.00 a | T a | T a | 2.12 a | 2.20 a | 1.22 a | T a | 2.23 b | 21.00 a | T a | T a | 1.42 a | 0.60 a | 2.14 a | 1.60 a |
| 22.8° C | 4.26 a | 19.78 a | 1.23 a | 2.03 a | 4.70 a | 18.53 a | 1.66 a | 0.53 a | 3.67 a | 3.50 a | 1.54 a | 0.53 a | 4.92 a | 21.43 a | 2.57 a | 1.47 a | 3.50 a | 1.63 b | 4.51 a | 11.27 a |
| 9.8° C | 2.25 b | 2.80 b | 2.51 a | 5.72 a | 1.26 b | 0.96 b | 1.76 a | 3.00 a | 1.30 b | 7.04 a | 1.38 a | 3.48 a | 3.00 b | 12.40 b | 2.67 a | 3.12 a | 2.80 a | 8.76 a | 0.85 b | 3.12 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.
T = Trace.
1)Total area (Log of integrator unit values).
2)Percentage of total peak areas.

TABLE 8h. Chromatogram signatures for E. carotovora var. carotovora (ECC), E. carotovora var. atroseptica (ECA), and non-inoculated controls (CO) averaged over two incubation temperatures.

| | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| Trial 1 | | |
| ECA | K A C G F J I B D E : h | K A C J G F D I B E : h |
| ECC | I F A D G C K H E J B | F A K I C G D H E J : b |
| CO | A G J I B F K C D E H | A I K F C J : bdeh |
| Trial 2 | | |
| ECA | A G C I H E K D F B : j | A G C K I F E H D B : j |
| ECC | G A I K H E C B F D J | G C E K A I H D F B J |
| CO | G C K F E I : abdhj | G C E K I : abdfhj |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

TABLE 8i. Comparisons between signatures for E. carotovora var. carotovora (ECC), E. carotovora var. atroseptica (ECA), and non-inoculated control (CO) treatments averaged over two incubation temperatures.

| | Characters Distinguishing Between Treatments[a] | | | |
|---|---|---|---|---|
| | ECC | | ECA | |
| Areas[b] | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| ECC | . | . | DHI | * |
| CO | CDHI | AG | C | ABG |
| PTPA[c] | | | | |
| ECC | . | . | F | A |
| CO | DF | * | G | A |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

* No characters significantly different.

TABLE 8j. Chromatogram signatures, averaged for the disease treatments at two incubation temperatures.

| | Signature[a] for area[b] | Signature for PTPA[c] |
|---|---|---|
| Trial 1 | | |
| 22.8° C | A F I C D K G J H B E | A F C K I D G J B H E |
| 9.8° C | G A K F J I C B E D : h | K G A I J C F D B E : h |
| Trial 2 | | |
| 22.8° C | G C K A E I H D F B J | G A C K E I H F D B J |
| 9.8° C | G I H A D C F E K B : j | G I E F C H A K D B : j |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

TABLE 8k. Comparisons between signatures averaged for the disease treatments at two incubation temperatures.

| | Characters Distinguishing Between Treatments[a] | | | |
|---|---|---|---|---|
| | 9.8° C | | | |
| | Areas[b] | | PTPA[c] | |
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| 22.8° C | BCDFHI | ACEGK | BCFG | ACGI |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

significantly different peak production and PTPA values for a wide range of metabolites. In the peak area comparisons, the 22.8° C values were usually significantly greater than at 9.8° C. However, only ethanol (peak area and PTPA 22.8 > 9.8° C) was a consistent diagnostic factor in the two trials.

The peak areas ($LOG_{10}$) and PTPA values, averaged over the incubation period for the major peaks in the chromatograms of the six experimental treatments are listed in Table 81. Table 8m contains the chromatogram signatures for the mean volatile output and PTPA values. The metabolites which, according to the test statistics in Table 81 could potentially be used to differentiate between the various treatments, are summarized in Table 8n. Very few metabolites were found to be diagnostic factors in both replicates of any of the paired comparisons. Again in peak area comparisons the disease treatment values tended to be greater than those of the controls and the 22.8° C values were usually significantly greater than at 9.8° C.

TABLE 81. Areas of chromatogram peaks averaged over the incubation period for E. carotovora var. carotovora (ECC) and E. carotovora var. atroseptica (ECA) incubated at two temperatures.

| | Methanol | | Ethanal | | Ethanol | | 2-Propanol | | RT = 7.1 min | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| Trial 1 | | | | | | | | | | | | | | | | | | | | |
| ECA 22.8° C | 5.10 a* | 28.80 a | T a | T a | 3.87 ab | 10.10 ab | 3.24 ab | 0.60 a | 1.60 a | 0.10 a | 3.60 a | 4.00 b | 3.28 a | 1.30 b | T b | T a | 3.98 a | 2.60 a | 3.69 a | 1.20 a |
| ECC 22.8° C | 5.04 a | 13.47 a | 0.99 a | 0.07 a | 5.01 a | 16.47 a | 4.73 a | 4.20 a | T a | T a | 5.46 a | 21.33 a | 3.47 a | 0.53 b | 2.68 a | 0.33 a | 4.45 a | 7.20 a | 3.43 a | 9.33 a |
| CO 22.8° C | 4.33 a | 17.80 a | T a | T a | 2.07 bc | 0.30 ab | 2.16 bc | T a | T a | T a | 3.65 a | 4.40 b | 3.04 a | 2.00 b | T b | T a | 4.01 a | 7.20 a | 3.17 a | 5.00 a |
| ECA 9.8° C | 3.53 a | 10.80 a | 2.60 a | 5.40 a | 3.73 ab | 7.13 ab | 0.92 bc | 3.20 a | 2.42 a | 0.93 a | 2.88 ab | 1.87 b | 3.79 a | 14.40 a | T b | T a | 0.99 b | 2.20 a | 4.64 a | 32.83 a |
| ECC 9.8° C | 2.72 a | 10.53 a | 3.34 a | 10.87 a | 0.98 c | 1.20 ab | 2.60 ab | 3.47 a | 1.02 a | 0.20 a | 2.41 ab | 5.73 b | 3.47 a | 14.60 a | T b | T a | 3.81 a | 11.80 a | 1.66 a | 12.80 a |
| CO 9.8° C | 3.79 a | 16.60 a | 1.05 a | 0.40 a | T c | T b | T c | T a | 1.86 a | T a | T b | T b | 3.40 a | 2.60 b | T b | T a | T b | T a | T a | T a |
| Trial 2 | | | | | | | | | | | | | | | | | | | | |
| ECA 22.8° C | 5.14 a | 33.80 a | T b | T a | 4.61 a | 16.07 ab | 3.32 a | 1.07 a | 3.77 a | 1.73 a | 1.34 a | 1.07 a | 4.42 ab | 6.53 c | 3.63 a | 2.60 a | 3.81 a | 1.46 ab | 4.46 a | 12.80 b |
| ECC 22.8° C | 5.08 a | 8.60 b | 1.85 ab | 1.20 a | 5.28 a | 30.00 a | T a | T a | 4.29 a | 6.80 a | 1.46 a | T a | 5.86 a | 44.00 a | 2.27 a | 0.50 a | 4.06 a | 2.40 ab | 5.09 a | 13.80 b |
| CO 22.8° C | T c | T b | 3.70 a | 9.80 a | 3.82 ab | 3.00 b | T a | T a | 2.12 a | 2.20 a | 1.32 a | T a | 4.53 ab | 21.00 b | T a | T a | 1.42 a | 0.60 b | 3.52 a | 1.60 b |
| ECA 9.8° C | 3.29 ab | 3.10 b | 1.14 ab | 1.80 a | 2.34 b | 2.20 b | 1.50 a | 0.60 a | 1.40 a | 1.70 a | 1.81 a | 6.30 a | 3.91 ab | 23.00 b | 2.70 a | 0.40 a | 3.83 a | 12.00 a | T a | T b |
| ECC 9.8° C | 1.56 bc | 2.60 b | 3.43 a | 8.33 a | 0.53 c | 0.13 b | 1.93 a | 4.60 a | 1.23 a | 10.60 a | 1.10 a | 1.60 a | 2.40 b | 5.33 c | 2.65 a | 4.93 a | 2.11 a | 6.60 ab | 1.43 a | 5.20 b |
| CO 9.8° C | T c | T b | 2.93 ab | 4.77 a | 2.75 b | 3.15 b | 3.23 a | 9.41 a | 2.94 a | 4.91 a | T a | T a | 3.31 b | 11.27 bc | T a | T a | 3.13 a | 7.47 ab | 3.59 a | 21.66 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.
T = Trace.

[1]Total area (Log of integrator unit values).

[2]Percentage of total peak areas.

TABLE 8m. Chromatogram signatures for E. carotovora var. atroseptica (ECA), E. carotovora var. carotovora (ECC), and non-inoculated controls (CO) at two incubation temperatures.

| | Signature[a] for area[b] | Signature for PTPA[c] |
|---|---|---|
| **Trial 1** | | |
| ECA 22.8° C | A K I C F D G J E B : h | A C F K I D B G J E : h |
| ECC 22.8° C | F A C D I G K H J B : e | F C A K I D G H J : be |
| CO 22.8° C | A I F K G J D C : beh | A I K F G C J : bdeh |
| ECA 9.8° C | K G C J A F B E I D : h | K G J A C D I F B E : h |
| ECC 9.8° C | I G A D F K E C : bhj | G K I A F D C E : bhj |
| CO 9.8° C | B A J G C E : dfhik | A G : bcdefhijk |
| **Trial 2** | | |
| ECA 22.8° C | A C K G I E H D F : bj | A C K G H E I F D : bj |
| ECC 22.8° C | G C K A E I B J H F : d | G C K A E I H B J : df |
| CO 22.8° C | G C K F E I : abdhj | G C E K I : abdfhj |
| ECA 9.8° C | G I A C H F D B E K : j | G I C F A E D H B K : j |
| ECC 9.8° C | H G I D A K E F C : bj | E I G K H D A F C : bj |
| CO 9.8° C | K I G D E B C : afhj | K G I D E B C : afhj |

[a] Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

[b] Log peak areas.

[c] Percentage of total peak areas.

TABLE 8n. Comparisons between signatures for E. carotovora var. carotovora (ECC), E. carotovora var. atroseptica (ECA), and non-inoculated control (CO) treatments at two incubation temperatures.

| | Characters Distinguishing Between Treatments[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ECA 22.8° C | | ECC 22.8° C | | CO 22.8° C | | ECA 9.8° C | | ECC 9.8° C | |
| Areas[b] | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| ECA 22.8° C | | | | | | | | | | |
| ECC 22.8° C | H | D | | | | | | | | |
| CO 22.8° C | * | ABDH | CDH | A | | | | | | |
| ECA 9.8° C | I | C | DHI | C | I | A | | | | |
| ECC 9.8° C | C | ABC | CH | ACG | * | C | CDI | C | | |
| CO 9.8° C | CDFI | AC | CDFHI | ACG | FI | * | * | A | CDI | C |
| PTPA[c] | | | | | | | | | | |
| ECA 22.8° C | | | | | | | | | | |
| ECC 22.8° C | F | AG | | | | | | | | |
| CO 22.8° C | * | AG | F | CG | | | | | | |
| ECA 9.8° C | G | AG | FG | CG | G | I | | | | |
| ECC 9.8° C | G | A | FG | CG | G | G | * | G | | |
| CO 9.8° C | C | AK | CFK | CGK | * | K | G | K | G | K |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

*No characters significantly different.

## Experiment 9: Effect of Inoculum Size on Volatile Production and Temperature Effects on Trap Performance

Disease summary. In both trials, disease development amongst the treatment replicates appeared to be relatively uniform. There were no appreciable differences between the patterns or rates of disease development in the large and small inoculum-size treatments. All control treatments remained sound throughout the test period.

E. carotovora bacteria were recovered from the bacterial seepage of all inoculated treatments. Tests indicated that both varieties of E. carotovora bacteria were present at the end of the incubation period in all inoculated replicates. In both trials, the populations of secondary microflora were relatively low in comparison with the E. carotovora populations. In Trial 1, E. coli, Staphylococcus, and Clostridium were the predominant secondary microorganisms. In Trial 2, yeasts and E. coli were predominant. The final populations of E. carotovora appeared to be relatively uniform regardless of the initial inoculum size.

Volatile Production. The analysis of variance statistics for the total volatile outputs of the various experimental treatments, averaged over the entire test period, are presented in Tables 9a and b. Over the two trials, the output from the diseased treatment averaged 25X the output of the controls (Table 9b). In both trials the large inoculum (LI) treatments produced significantly ($P = 0.05$) greater average volatile outputs than the small inoculum (SI) treatments. Over the two trials, the average output of the LI treatments was approximately 3X greater than for the SI treatments (Table 9b). In both experimental trials, the trap temperature main effect was not significant ($P = 0.05$) (Table 9a). All two- and three-way interactions were nonsignificant.

TABLE 9a. Analysis of variance for means of total of peak areas[1] averaged over the incubation period for a mixed E. carotovora infection with two inoculum sizes and two trap temperatures.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Trial 1 | | | | |
| Disease (D) | 1 | 6.32 | 6.32 | 41.11** |
| Inoc. size (I) | 1 | 1.18 | 1.18 | 7.67* |
| Trap temp. (T) | 1 | 0.06 | 0.06 | 0.39N.S. |
| DXI | 1 | 0.68 | 0.68 | 4.53N.S. |
| DXT | 1 | 0.41 | 0.41 | 2.67N.S. |
| IXT | 1 | 0.49 | 0.49 | 3.19N.S. |
| DXIXT | 1 | 0.32 | 0.32 | 2.13N.S. |
| Error | 8 | 1.23 | 0.15 | |
| Total | 15 | 10.69 | | |
| Trial 2 | | | | |
| Disease (D) | 1 | 8.85 | 8.85 | 177.00** |
| Inoc. size (I) | 1 | 0.41 | 0.41 | 8.20* |
| Trap temp. (T) | 1 | 0.09 | 0.09 | 1.80N.S. |
| DXI | 1 | 0.04 | 0.04 | 0.80N.S. |
| DXT | 1 | 0.26 | 0.26 | 5.20N.S. |
| IXT | 1 | 0.01 | 0.01 | 0.20N.S. |
| DXIXT | 1 | 0.121 | 0.121 | 2.42N.S. |
| Error | 8 | 0.39 | 0.05 | |
| Total | 15 | 10.16 | | |

** Significant at the 1% level.

* Significant at the 5% level.

N.S. Not significant at the 5% level.

1) Log of sum of all individual peak areas.

TABLE 9b. Means of total daily integrator responses[1)] averaged over the incubation period for a mixed E. carotovora infection with two inoculum sizes and two trap temperatures.

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Treatment | Means | Treatment | Means |
| $\bar{X}$ Diseased | 5.59 a* | $\bar{X}$ Diseased | 6.08 a |
| $\bar{X}$ Control | 4.33 b | $\bar{X}$ Control | 4.59 b |
| $\bar{X}$ Large inoc. | 5.27 a | $\bar{X}$ Large inoc. | 5.49 a |
| $\bar{X}$ Small inoc. | 4.66 b | $\bar{X}$ Small inoc. | 5.17 b |
| $\bar{X}$ 1° C Trap | 5.02 a | $\bar{X}$ 1° C Trap | 5.41 a |
| $\bar{X}$ 22° C Trap | 4.90 a | $\bar{X}$ 22° C Trap | 5.25 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test for ordered means.

1)Log of sum of all individual peak areas.

The regression coefficients for the exponential regression equations (Table 9c) indicate that in Trial 1 the total daily volatile outputs of both the diseased and the control treatments followed an exponential pattern of increase over the incubation period. The total daily volatile outputs of the inoculum size treatments in Trial 1 also increased exponentially over the duration of the test (Table 9c). Covariant analysis indicated that the diseased and control treatments in Trial 1 had significantly different regression slopes ($P = 0.01$) and general regression equations ($P = 0.01$) (Table 9d). In the inoculum size analysis, the regression lines were found to have significantly different slopes ($P = 0.01$), but the regression equations were not significantly different ($P = 0.05$). The total volatile outputs of the disease-type and inoculum-size treatments in Trial 2 followed the same general pattern as in Trial 1, but were much more variable.

In both trials, all recorded chromatogram peaks were common to the volatile profiles of both the inoculum-size treatments (REFERENCE TABLE C). The analysis of variance statistics for the mean number of peaks/chromatogram, averaged over the entire incubation period, are presented in Tables 9e and f. In both trials, the disease variable was the only main plot factor to show statistically significant differences between the treatments. In both trials, the E. carotovora infected treatment exhibited a significantly ($P = 0.01$) greater average number of peaks/chromatogram than the control treatment. All two- and three-way interactions were non-significant.

The peak areas ($LOG_{10}$) and PTPA values, averaged over the entire incubation period, for the major peaks in the chromatograms of the disease treatments, the inoculum-size treatments, and the trap-temperature treatments are listed in Table 9g. Tables 9h, i, and j contain the chromatogram signatures for the mean peak areas and PTPA values (see REFERENCE TABLE D for peak identificatio

TABLE 9c. Linear regression equations for the daily total integrator responses for a mixed E. carotovora infection with two inoculum sizes.

| Treatment | Equation | $r^2$ |
|---|---|---|
| $\bar{X}$ Diseased | $Y^{1)} = e^{8.37+0.90\bar{X}^{2)}}$ | $0.54^{**}$ |
| $\bar{X}$ Control | $Y = e^{3.24+0.45\bar{X}}$ | $0.63^{*}$ |
| $\bar{X}$ Large inoc. | $Y = e^{8.06+1.22\bar{X}}$ | $0.76^{**}$ |
| $\bar{X}$ Small inoc. | $Y = e^{8.67+0.59\bar{X}}$ | $0.81^{**}$ |

1) Y = Total integrator response (integrator units).
2) $\bar{X}$ = Incubation period (days)
** Significant at the 1% level.
* Significant at the 5% level.

TABLE 9d. Analysis of covariance for the linear regression equations calculated for the daily total integrator responses[1)] for a mixed E. carotovora infection with two inoculum sizes.

| Pairwise comparison | F test for slope | F test for similarity between lines |
|---|---|---|
| Diseased vs Control | $9.77^{**}$ | $20.75^{**}$ |
| Large inoc. vs Small inoc. | $30.67^{**}$ | $2.56^{N.S.}$ |

** Difference significant at the 1% level.
N.S. Difference not significant at the 5% level.
1) Ln of integrator units.

TABLE 9e. Analysis of variance for mean number of peaks/chromatogram averaged over the incubation period for a mixed E. carotovora infection with two inoculum sizes and two trap temperatures.

| Source of variation | D.F. | S.S. | M.S. | F-ratio |
|---|---|---|---|---|
| Trial 1 | | | | |
| Disease (D) | 1 | 18.49 | 18.49 | $14.01^{**}$ |
| Inoc. size (I) | 1 | 4.95 | 4.95 | $3.75^{N.S.}$ |
| Trap temp. (T) | 1 | 1.51 | 1.51 | $1.14^{N.S.}$ |
| DXI | 1 | 2.60 | 2.60 | $1.97^{N.S.}$ |
| DXT | 1 | 0.55 | 0.55 | $0.42^{N.S.}$ |
| IXT | 1 | 0.01 | 0.01 | $0.01^{N.S.}$ |
| DXIXT | 1 | 0.31 | 0.31 | $0.23^{N.S.}$ |
| Error | 8 | 10.58 | 1.32 | |
| Total | 15 | 39.00 | | |
| Trial 2 | | | | |
| Disease (D) | 1 | 46.58 | 46.58 | $60.20^{**}$ |
| Inoc. size (I) | 1 | 1.16 | 1.16 | $1.50^{N.S.}$ |
| Trap temp. (T) | 1 | 2.48 | 2.48 | $3.20^{N.S.}$ |
| DXI | 1 | 0.33 | 0.33 | $0.43^{N.S.}$ |
| DXT | 1 | 12.71 | 12.71 | $16.43^{N.S.}$ |
| IXT | 1 | 0.14 | 0.14 | $0.18^{N.S.}$ |
| DXIXT | 1 | 2.18 | 2.18 | $2.82^{N.S.}$ |
| Error | 8 | 6.19 | 0.77 | |
| Total | 15 | 71.81 | | |

$^{**}$Significant at the 1% level.

$^{N.S.}$Not significant at the 5% level.

TABLE 9f. Mean number of peaks/chromatogram averaged over the incubation period for a mixed *E. carotovora* infection with two inoculum sizes and two trap temperatures.

| Treatment | Means | |
|---|---|---|
| | Trial 1 | Trial 2 |
| $\bar{X}$ Diseased | 6.4 a* | 9.4 a |
| $\bar{X}$ Control | 4.2 b | 6.0 b |
| $\bar{X}$ Large inoc. | 5.8 a | 8.0 a |
| $\bar{X}$ Small inoc. | 4.7 a | 7.4 a |
| $\bar{X}$ 1° C Trap | 5.6 a | 8.1 a |
| $\bar{X}$ 22° C Trap | 5.0 a | 7.3 a |

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Tukey's test for ordered means.

TABLE 9g. Areas of chromatogram peak averaged over the incubation period for a mixed E. carotovora infection with two inoculum sizes and two trap temperatures.

| | Methanol | | Ethanal | | Ethanol | | 1-Propanal/ 2-Propanone | | RT = 7.1 min | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy- 2-Butanone | | RT = 17.8 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| **Trial 1** | | | | | | | | | | | | | | | | | | | | |
| Diseased | 3.15 a* | 1.19 a | 4.41 a | 8.46 a | 4.57 a | 34.19 a | 0.89 a | T a | 3.41 a | 5.08 a | 2.72 a | 0.41 a | 4.31 a | 4.47 b | 3.50 a | 6.33 a | 3.67 a | 8.58 a | 3.74 a | 0.42 a |
| vs | | | | | | | | | | | | | | | | | | | | |
| Control | 0.85 a | 4.13 a | 0.56 b | 2.75 a | 1.61 b | 4.13 b | T a | T a | 1.96 b | 1.04 a | 0.69 b | 2.04 a | 0.70 b | 6.60 a | 3.30 a | 9.74 a | 2.27 b | 12.81 a | 0.93 b | 5.79 a |
| L. Inoc. | 2.66 a | 6.67 a | 2.89 a | 3.69 a | 4.12 a | 33.72 a | 0.64 a | 0.03 a | 3.59 a | 4.03 a | 2.65 a | 1.97 a | 3.88 a | 6.00 a | 3.54 a | 9.66 a | 2.82 | 9.20 a | 2.93 a | 2.72 a |
| vs | | | | | | | | | | | | | | | | | | | | |
| S. Inoc. | 2.10 a | 4.33 a | 2.80 a | 9.42 a | 3.05 a | 14.61 a | 0.56 a | 1.50 a | 2.26 b | 0.75 a | 1.43 a | 1.03 a | 2.33 a | 3.11 a | 3.33 a | 15.97 a | 3.61 | 13.61 a | 2.68 a | 5.28 a |
| 1° C | 2.41 a | 6.73 a | 2.50 a | 5.45 a | 3.97 a | 29.09 a | 0.55 a | 0.02 a | 3.33 a | 4.07 a | 2.12 a | 0.40 a | 2.94 a | 2.95 a | 3.41 a | 11.90 a | 3.48 a | 12.36 a | 3.32 a | 5.77 a |
| vs | | | | | | | | | | | | | | | | | | | | |
| 22.8° C | 1.96 a | 3.14 a | 3.14 a | 6.86 a | 2.89 a | 15.08 a | 0.66 a | 1.50 a | 2.48 a | 1.25 a | 1.61 a | 2.53 a | 2.78 a | 5.67 a | 3.31 a | 14.08 a | 2.95 a | 13.81 a | 1.96 a | 1.90 a |
| **Trial 2** | | | | | | | | | | | | | | | | | | | | |
| Diseased | 4.79 a | 4.88 a | 0.61 a | T a | 3.03 a | 15.13 a | 1.11 a | 0.03 b | 4.22 a | 4.09 a | 3.56 a | 0.53 a | 5.32 a | 46.25 a | 3.15 a | 0.88 b | 4.20 a | 4.66 a | 4.19 a | 1.41 a |
| vs | | | | | | | | | | | | | | | | | | | | |
| Control | 3.13 b | 3.25 a | T a | T a | 2.95 a | 4.50 a | 1.56 a | 1.25 a | 2.49 b | 0.75 a | T a | T a | 3.74 b | 10.75 b | 3.74 a | 16.75 a | 3.69 a | 8.50 a | T b | T a |
| L. Inoc. | 4.80 a | 3.75 a | 1.22 a | T a | 4.52 a | 11.63 a | 1.25 a | T a | 4.31 a | 4.63 a | 4.19 a | 2.69 a | 5.16 a | 45.31 a | 2.92 a | 1.31 a | 4.58 a | 5.06 a | 3.97 a | 1.25 a |
| vs | | | | | | | | | | | | | | | | | | | | |
| S. Inoc. | 4.78 a | 6.00 a | T a | T a | 3.53 a | 18.63 a | 0.98 a | 0.06 a | 4.14 a | 13.56 a | 2.97 a | 0.38 a | 5.04 b | 47.18 a | 3.38 a | 0.44 a | 3.82 a | 4.25 a | 3.41 a | 1.67 a |
| 1° C | 4.49 a | 3.60 a | 0.45 a | T a | 4.50 a | 14.20 a | 2.09 a | 0.30 a | 3.90 a | 3.00 a | 2.33 a | 0.10 b | 4.88 b | 39.75 a | 3.84 a | 3.55 a | 3.93 a | 6.25 a | 3.22 b | 0.65 b |
| vs | | | | | | | | | | | | | | | | | | | | |
| 22.8° C | 4.76 a | 6.06 a | 0.66 a | T a | 5.42 a | 13.63 a | T a | T b | 4.20 a | 4.63 a | 4.25 a | 0.94 a | 5.48 a | 45.50 a | 3.69 a | 1.50 b | 4.41 a | 3.63 a | 4.35 a | 2.00 a |

T = Trace.

1) Total area (Log of integrator units).

2) Percentage of total peak areas.

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test of ordered means.

TABLE 9h. Chromatogram signatures for E. carotovora infected and non-inoculated control treatments.

| | Trial 1 | |
|---|---|---|
| | Signature[a] for areas[b] | Signature for PTPA[c] |
| Diseased | C G K I H E A B F J D | C I H B G A K F E J : d |
| Control | H K I B E C A G F : dj | H I K A E C B G F : dj |
| | **Trial 2** | |
| | Signature for areas | Signature for PTPA |
| Diseased | G A E I K F H C J D B | G C A I E K H F J D : b |
| Control | I H G K A C E D B F : j | I K H G C D A E B : fj |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same brackets. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 9i. Chromatogram signatures for large and small inoculum size treatments.

| | Trial 1 | |
|---|---|---|
| | Signature[a] for areas[b] | Signature for PTPA[c] |
| Large inoculum | C G E H K B I A F J : d | C H I A G E K B F J : d |
| Small inoculum | I H C B K G E A F J D | H C I B K A G F E : dj |
| | **Trial 2** | |
| | Signature for areas | Signature for PTPA |
| Large inoculum | G A I E K F H C J D : b | G C I E A K H F J : bd |
| Small inoculum | G A K E I C H F D J : b | G C A I E K H F D : bj |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 9j. Chromatogram signatures for trap temperature treatments.

| | Trial 1 | |
|---|---|---|
| | Signature[a] for areas[b] | Signature for PTPA[c] |
| 1.0° C | C I H E G B A F K J : d | C I H A B E G K F : dj |
| 22.8° C | K H B I C G E A F J D | C I H B K G A F E J : d |
| | **Trial 2** | |
| | Signature for areas | Signature for PTPA |
| 1° C | G A I E K C H F D J B | G C K I A H E D F : bj |
| 22.8° C | G A I F K E H C J B D | G C I A H E K D F B J |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

The metabolites which, according to the test statistics in Table 9g could potentially be used to differentiate between the chromatograms for each treatment, are summarized in Tables 9k, l, and m.

In the disease treatment analysis, the peak areas of a number of metabolites were found to be significantly different between the E. carotovora infected and control treatments. 1-Propanol, 1-butanol, and compound K were found to be consistent diagnostic factors in both trials. In all cases, the average output of these compounds by the diseased treatments was significantly greater than that of the control treatments. Only 1-butanol was a consistent diagnostic factor in the PTPA values of the two trials. However, in Trial 1, 1-butanol was of greater relative importance in the control treatment, while in Trial 2, the PTPA value for 1-butanol in the diseased treatment was significantly greater than in the control treatment.

In the inoculum-size analysis, there were few significant differences between the volatile fingerprints for the two inoculum sizes.

In the trap temperature analysis, there were relatively few diagnostic factors and the degree of correspondence between the results in the two experimental replicates was low.

TABLE 9k. Comparisons between signatures for E. carotovora infected and non-inoculated control treatments.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | Control | |
| Areas[b] | Trial 1 | Trial 2 |
| Diseased | BCEFGIK | AEGK |
| PTPA[c] | | |
| Diseased | CG | DGH |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 91. Comparisons between signatures for large and small inoculum size treatments.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | Small Inoculum | |
| Area[b] | Trial 1 | Trial 2 |
| Large inoculum | E | F |
| PTPA[c] | Small Inoculum | |
| Large inoculum | * | * |

[a] Characters which differ significantly between the treatments.

[b] Log peak areas.

[c] Percentage of total peak areas.

* No characters different.

TABLE 9m. Comparisons between signatures for trap temperature treatments.

| | Characters Distinguishing Between Treatments[a] | |
|---|---|---|
| | 1° C | |
| Areas[b] | Trial 1 | Trial 2 |
| 22.8° C | * | GK |
| PTPA[c] | 1° C | |
| 22.8° C | * | DFHK |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

* No characters different.

## Experiment 10: Volatile Profile of Ring Rot (C. sepedonicum) Infection

Disease summary. The rate and pattern of disease development amongst the C. sepedonicum inoculated replicates was relatively uniform. The first signs of disease became apparent on day 7 of the incubation period as the tubers began to crack and collapse. By day 13, the bulk of the tubers' vascular and cortical tissues had been destroyed by the pathogen. All control treatments remained sound throughout the incubation period.

At the end of the incubation period, C. sepedonicum bacteria were isolated from the bacterial seepage of all inoculated treatments. Plating of the seepage showed that C. sepedonicum was the predominant organism, however, populations of secondary microflora were large in comparison to the E. carotovora tests. Staphylococcus, E. coli, and yeasts were the predominant secondary microorganisms.

Volatile production. The total daily volatile output data from the C. sepedonicum and control treatments were plotted as an exponential function in Figure 10a, along with the daily output values from the mixed E. carotovora infection investigated in Experiment 4. The daily volatile output of the C. sepedonicum treatments increased steadily until day 9 of the incubation period and then declined through to day 13. By day 9, the daily volatile output of the C. sepedonicum treatments was 735X greater than on day 1 (Table 10a). At day 13, the volatile output remained at 75X that of day 1. The total daily volatile output of the C. sepedonicum treatments was consistently greater than that of the controls (Table 10a). Averaged over the entire incubation period the volatile output of the infected treatment was approximately 45X greater than for the controls, a difference significant to the 1% level (Table 10b).

FIGURE 10a. Daily total volatile production for C. sepedonicum, E. carotovora, and control treatments.

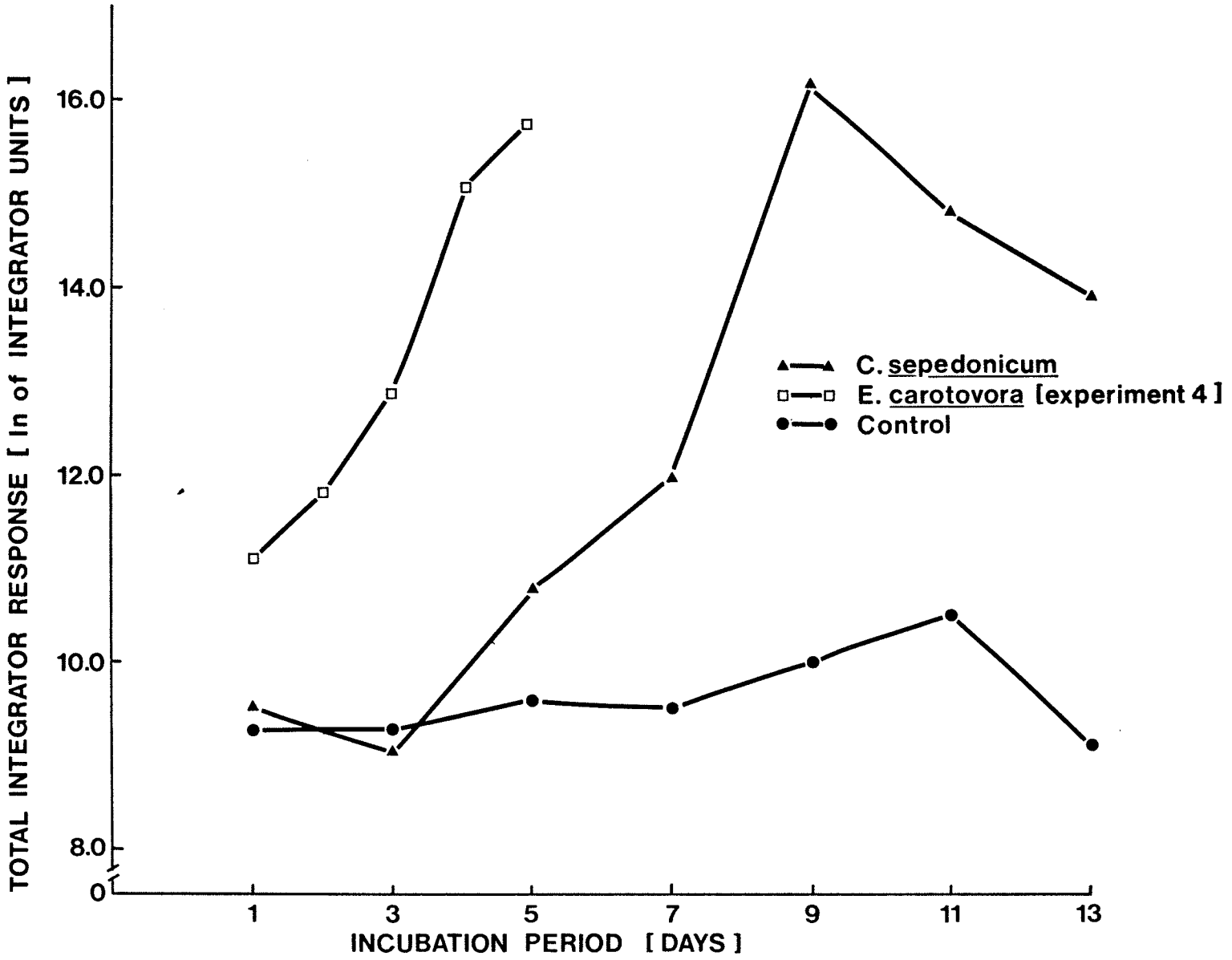

TOTAL INTEGRATOR RESPONSE [ ln of INTEGRATOR UNITS ]
16.0
14.0
12.0
10.0
8.0
0
1
3
5
7
9
11
13
INCUBATION PERIOD [ DAYS ]
C. sepedonicum
E. carotovora [experiment 4 ]
Control

TABLE 10a. Relative total peak areas[1] for C. sepedonicum experiment.

| Treatment | Incubation Period (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 |
| | % | | | | | | |
| C. sepedonicum | 0.1[2] | 0.1 | 0.4 | 1.3 | 100.0 | 21.2 | 14.4 |
| Control | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 |

1) C. sepedonicum at day 9 = 100.

2) Values = peak area/peak area C. sepedonicum day 9 X 100.

TABLE 10b. Pairwise comparisons of means of total peak areas[1] averaged over the incubation period for C. sepedonicum, E. carotovora, and control treatments.

| Pairwise comparison | Mean ($LOG_{10}$) total integrator response | t Value |
|---|---|---|
| C. sepedonicum (day 1-13) | 6.46 | 9.49** |
| vs | | |
| Control (day 1-13) | 4.80 | |
| C. sepedonicum (day 1-5) | 4.31 | 7.51** |
| vs | | |
| E. carotovora (day 1-5) | 6.36 | |
| C. sepedonicum (day 1-13) | 6.46 | 0.33N.S. |
| vs | | |
| E. carotovora (day 1-5) | 6.36 | |

** Values significantly different at the 1% level.

N.S. Values not significantly different at the 5% level.

1) Log of sum of all individual peak areas.

In the C. sepedonicum treatments, the number of peaks/chromatogram increased steadily over the duration of the test period (Table 10c). The mean number of peaks/chromatogram in the C. sepedonicum treatments was not significantly (P = 0.05) different from the controls (Table 10d).

Linear regression equations were derived for the experimental treatments, based upon the natural logarithms of the daily total volatile output values (Table 10e). Additionally, the natural logs of the C. sepedonicum data were fitted to a parabolic regression model. Although the C. sepedonicum data did fit a straight-line equation (P = 0.01), the fit to the parabolic equation was more accurate (P = 0.01).

Covariant analysis indicated that the slope and regression equation derived for the C. sepedonicum treatments were significantly (P = 0.01) different from the equation derived for the control treatments (Table 10f).

Schematic chromatograms representing the peak areas and PTPA values at three points during the disease cycles of a C. sepedonicum and a mixed variety E. carotovora infection are presented in Figure 10b. The volatile profiles could be differentiated on the basis of a number of factors.

Based on the data for days 1 to 5 of the incubation period, the total volatile output of the E. carotovora treatment was, on the average, 112X greater than that for the C. sepedonicum treatment (Table 10b); a difference significant at the 1% level. When the data for the entire 13 day incubation period of the C. sepedonicum treatment was included in the analysis there was no significant difference (P = 0.05) between the average total volatile outputs of the E. carotovora and C. sepedonicum treatments.

A linear regression equation was also derived for the C. sepedonicum treatments based on the natural logs of the total volatile output data from days 1 to 5 (Figure 10a). This enabled comparisons to be made with the

TABLE 10c. Number of peaks/chromatogram for *C. sepedonicum* experiment.

| Treatment | Incubation Period (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 |
| *C. sepedonicum* | 5 | 6 | 5 | 9 | 11 | 13 | 16 |
| Control | 6 | 6 | 7 | 6 | 9 | 12 | 11 |
| *E. carotovora* (Expt 4) | 10 | 8 | 11 | | | | |

TABLE 10d. Pairwise comparison of mean number of peaks/ chromatogram averaged over the incubation period for C. sepedonicum, E. carotovora, and control treatments.

| Pairwise comparison | Mean number of peaks/ chromatogram | t Value |
|---|---|---|
| C. sepedonicum (day 1-13) | 9.4 | |
| vs | | $1.46^{N.S.}$ |
| Control (day 1-13) | 8.1 | |
| C. sepedonicum (day 1-5) | 5.3 | |
| vs | | $7.61^{**}$ |
| E. carotovora (day 1-5) | 10.6 | |
| C. sepedonicum (day 1-13) | 9.4 | |
| vs | | $1.27^{N.S.}$ |
| E. carotovora (day 1-5) | 10.6 | |

**Values significantly different at the 1% level.

N.S. Values not significantly different at the 5% level.

TABLE 10e. Linear regression equations for the daily total integrator responses of C. sepedonicum, E. carotovora, and control treatments.

| Treatment | Equation | $r^2$ |
|---|---|---|
| C. sepedonicum (day 1-5) | $Y^{1)} = e^{8.84+0.35\bar{X}^{2)}}$ | $0.52^{N.S.}$ |
| C. sepedonicum (day 1-9) | $Y = e^{7.39+0.83\bar{X}}$ | $0.89^{**}$ |
| C. sepedonicum (day 1-13) | $Y = e^{7.98+0.55\bar{X}}$ | $0.68^{**}$ |
| | $Y = e^{7.50+0.97\bar{X}-0.03\bar{X}^2}$ | $0.79^{**}$ |
| Control (day 1-13) | $Y = e^{9.29+0.04\bar{X}}$ | $0.15^{N.S.}$ |
| E. carotovora (Expt 4) (day 1-5) | $Y = e^{9.66+1.24\bar{X}}$ | $0.85^{**}$ |

1) Y = Total integrator response (integrator units).

2) $\bar{X}$ = Incubation period (days).

** Significant at the 1% level.

N.S. Not significant at the 5% level.

TABLE 10f. Analysis of covariance for linear regression equations calculated for the total integrator responses[1)] of C. sepedonicum, E. carotovora, and control treatments.

| Pairwise comparison | F Test for slope | F Test for similarity between lines |
|---|---|---|
| C. sepedonicum vs Control | $11.74^{**}$ | $10.74^{**}$ |
| C. sepedonicum (day 1-5) vs E. carotovora (Expt 4) | $16.65^{**}$ | $18.55^{**}$ |
| C. sepedonicum (day 1-13) vs E. carotovora (Expt 4) | $10.69^{*}$ | $2.35^{N.S.}$ |

$^{**}$Difference significant at the 1% level.

$^{*}$Difference significant at the 5% level.

N.S. Difference not significant at the 5% level.

1) Ln of integrator units.

FIGURE 10b. Areas and PTPA* values for chromatogram peaks at three stages in C. sepedonicum and E. carotovora infections.

*Percentage of total peak areas.

Peak A = Methanol

B = Ethanal

C = Ethanol

D = ?

E = 1-Propanol

F = C-4 Compounds

G = 1-Butanol

H = ?

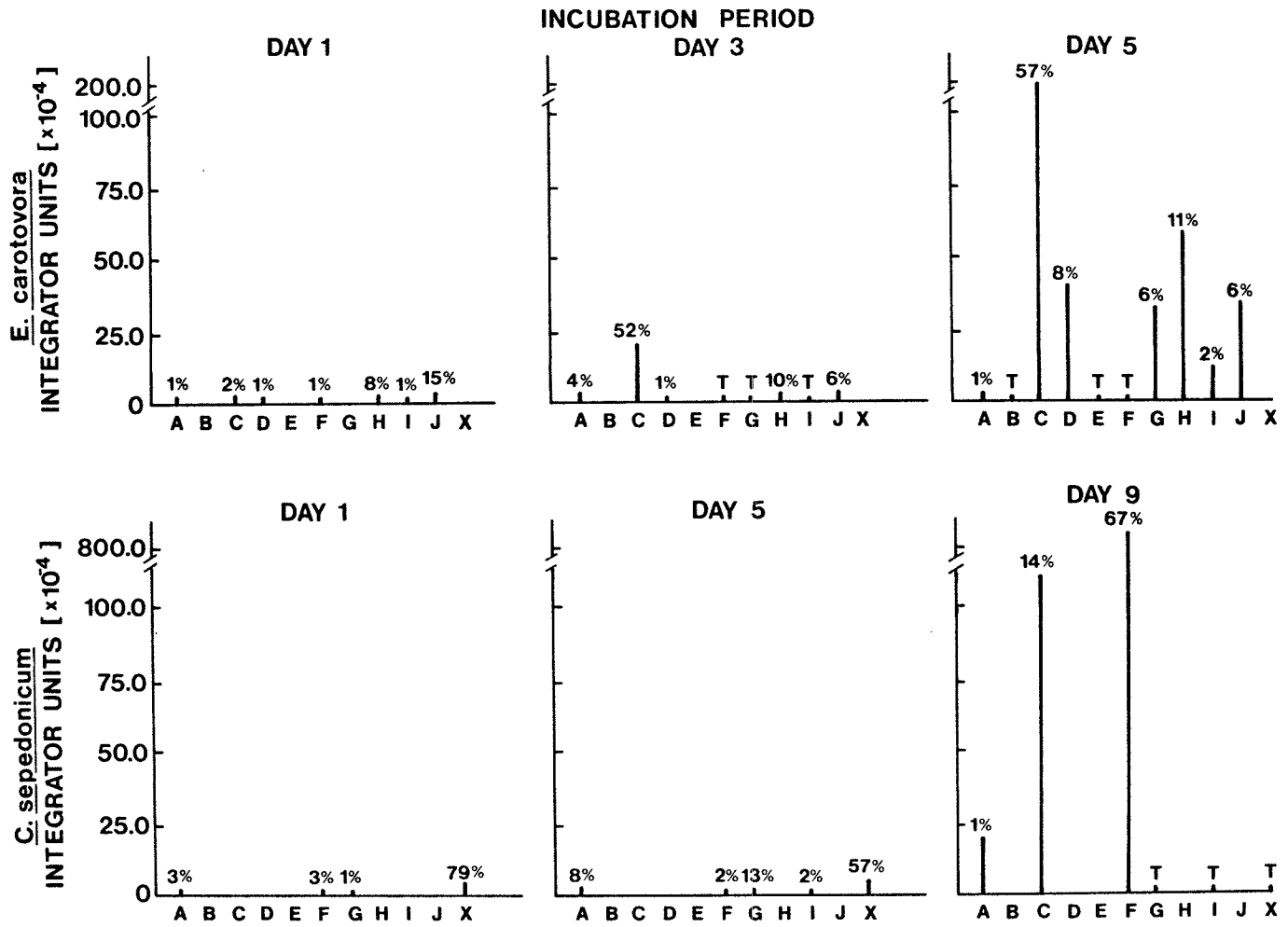
I = 3-Hydroxy-2-Butanone

J = ?

X = ? (RT = 15.6 min)

X Axis = Chromatogram peaks.

Y Axis = Peak areas.


INCUBATION PERIOD
DAY 1
DAY 3
DAY 5
E. carotovora
INTEGRATOR UNITS [×10⁻⁴]
200.0
100.0
75.0
50.0
25.0
0
1% 2% 1% 1% 8% 1% 15%
4% 52% 1% T T 10% T 6%
1% T 57% T T 6% 11% 2% 6%
A B C D E F G H I J X
DAY 1
DAY 5
DAY 9
C. sepedonicum
INTEGRATOR UNITS [×10⁻⁴]
800.0
100.0
75.0
50.0
25.0
0
3% 3% 1% 79%
8% 2% 13% 2% 57%
1% 14% 67% T T T
A B C D E F G H I J X

E. carotovora treatments over equivalent time frames. Based on the data from days 1 to 5, the slope and general equation for the C. sepedonicum regression line were significantly (P = 0.01) different from the values calculated for the E. carotovora treatment (Table 10f). Based on the data for days 1 to 13 in the C. sepedonicum trial, the slopes for the regression lines remained significantly (P = 0.05) different, however, the overall linear equations were not significantly different (P = 0.05).

The peak areas ($LOG_{10}$) and PTPA values, averaged over the entire incubation period, for the major peaks in the chromatograms of the mixed E. carotovora infection, the C. sepedonicum, and non-infected control treatments are listed in Table 10g. Separate analyses were conducted using the C. sepedonicum data from days 1 to 5 and from days 1 to 13. Table 10h contains the chromatographic signatures for the peak areas and PTPA values of the various treatments (see REFERENCE TABLE D for peak identification). Table 10i summarizes the metabolites which might be utilized to differentiate between the treatment profiles. When the C. sepedonicum and control data for days 1 to 5 were used, the treatments could be differentiated on the basis of the peak areas for ethanol (CO > CS), compound D (CO > CS), compound H (CO > CS), and compound X (CS > CO). The PTPA values for ethanol (CO > CS), 1-butanol (CS > CO), compound H (CO > CS), and compound X (CS > CO) were significantly different.

When the C. sepedonicum infection had fully developed, the peak areas for methanol, ethanol, compound D, 1-propanol, the C-4 compounds, and compounds H, K, and X were significantly different in the C. sepedonicum and control profiles. In all cases, the output treatments were significantly greater than the controls. The PTPA values for ethanol (CO > CS), the C-4 compounds (CS > CO), 1-butanol (CS > CO), and compounds H (CO > CS), K (CS > CO), and X (CS > CO) were significantly different.

TABLE 10g. Areas of chromatogram peaks, averaged over the incubation period for C. sapedonicum (CS), E. carotovora (EMX), and control (CO) treatments.

| | Methanol | | Ethanal | | Ethanol | | RT = 7.1 min | | 1-Propanol | | C-4 Pks | | 1-Butanol | | RT = 12.7 min | | 3-Hydroxy-2-Butanone | | RT = 17.8 min | | RT = 15.6 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Area[1] | PTPA[2] | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA | Area | PTPA |
| EMX | 4.50 a* | 3.53 b | 1.04 a | 0.06 a | 5.78 a | 37.65 a | 4.55 a | 2.51 a | 3.27 a | 0.01 a | 3.68 a | 1.46 a | 4.60 a | 2.09 b | 5.28 a | 11.11 a | 3.29 a | 2.11 a | 4.84 a | 8.57 a | T b | T |
| CS (day 1-5) | 3.17 ab | 13.83 a | T a | T a | T c | T b | T c | T a | 0.88 b | 0.33 a | T b | 3.58 a | 2.73 b | 6.83 a | T c | T b | 1.36 a | 1.17 a | 3.93 a | 22.92 a | 4.02 a | 41.43 |
| CO | 1.77 b | 11.17 ab | T a | T a | 4.43 b | 44.50 a | 3.03 b | 0.50 a | T b | T a | T b | T a | 2.85 b | 0.17 b | 4.44 b | 6.50 a | 3.39 a | 1.50 a | 4.69 a | 8.33 a | T b | T |
| EMX | 4.50 a | 3.53 b | 1.04 a | 0.06 a | 5.78 a | 37.65 a | 4.55 a | 2.51 a | 3.27 a | 0.01 a | 3.68 b | 1.46 b | 4.60 a | 2.09 b | 5.28 a | 11.11 a | 3.29 a | 2.11 a | 4.84 b | 8.57 b | T b | T a |
| CS (day 1-13) | 4.51 a | 15.00 a | T a | T a | 6.29 a | 20.50 b | T c | T a | 3.48 a | 0.36 a | 6.49 a | 47.61 a | 3.70 ab | 8.33 a | T c | T b | 3.68 a | 2.75 a | 5.25 a | 17.65 a | 4.16 a | T a |
| CO | 1.77 b | 11.17 a | T a | T a | 4.43 b | 44.50 a | 3.03 b | 0.50 a | T b | T a | T c | T b | 2.85 b | 0.17 b | 4.44 b | 6.50 a | 3.39 a | 1.50 a | 4.69 b | 8.33 b | T b | T a |

[1] Total area (Log of integrator units).

[2] Percentage of total peak areas.

* Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test for ordered means.

T = Trace.

TABLE 10h. Chromatogram signatures for a mixed E. carotovora infection, C. sepedonicum, and non-infected control treatments.

| Treatment | Signature[a] for areas[b] | Signature for PTPA[c] |
|---|---|---|
| C. sepedonicum (day 1-5) | X K A G F I E : bcdhj | X K A G F I E : bcdhj |
| C. sepedonicum (day 1-13) | F C A K E G I X : bdhj | F C A K E G I : bdhjx |
| E. carotovora | C H K G D A F I E B J | C H K G D A F I E B J |
| Control (day 1-13) | K C A H I D G F : bej | K C A H I D G F : bej |

a) Peaks significantly (P = 0.05) different from one another by Duncan's test for ordered means are not enclosed by the same bracket. Traces are given as small letters.

b) Log peak areas.

c) Percentage of total peak areas.

TABLE 10i. Comparisons between the chromatogram signatures for a mixed E. carotovora infection, C. sepedonicum, and non-infected control treatments.

| | Characters Distinguishing Between Treatments[a] | | | | | |
|---|---|---|---|---|---|---|
| | E. carotovora | | Control (day 1-5) | | Control (day 1-13) | |
| | Area[b] | PTPA[c] | Area | PTPA | Area | PTPA |
| C. sepedonicum (day 1-5) | CDEFGHX | ACGHX | CDHX | CGHX | . | . |
| C. sepedonicum (day 1-13) | CFHKX | ACFGHKX | . | . | ACDEFHKX | CFGHKX |

a) Characters which differ significantly between the treatments.

b) Log peak areas.

c) Percentage of total peak areas.

In comparing the C. sepedonicum day 1 to 5 data with the E. carotovora values the disease profiles could be differentiated on the basis of the peak areas for ethanol, 1-propanol, the C-4 compounds, 1-butanol, and compounds D, H, K, and X. With the exception of compound X (CS > EMX) the volatile production values for the E. carotovora infection were consistently greater than in the C. sepedonicum treatment. The PTPA values for methanol (CS > EMX), ethanol (EMX > CS), 1-butanol (CS > EMX), compound H (EMX > CS), and compound X (CS > EMX) were significantly different for the disease treatments.

When the data for days 1 to 13 were included in the C. sepedonicum analyses, the peak area values for compound D (EMX > CS), the C-4 compounds (CS > EMX), compound H (EMX > CS), K (CS > EMX), and X (CS > EMX) were significantly different for the disease treatments. The PTPA values for methanol (CS > EMX), ethanol (EMX > CS), the C-4 compounds (CS > EMX), 1-butanol (CS > EMX), compound H (EMX > CS), K (CS > EMX), and X (CS > EMX) could be used to differentiate between the disease treatments.

Experiment 11: Volatile Profiles of Bacterial Cultures

The concentration of volatiles in the headspace samples from the inoculated cultures increased by three to four times over the incubation period. The three different E. carotovora treatments (ECC, ECA, and EMX) produced relatively similar changes in the total concentration of volatiles above the cultures. The total concentration of volatiles in the non-inoculated cultures remained relatively stable over the incubation period.

The PTPA values for the major peaks in the chromatograms of the inoculated and non-inoculated cultures are presented in Table 11a.

TABLE 11a. PTPA[1)] values from headspace samples of E. carotovora var. atroseptica (ECA), E. carotovora var. carotovora (ECC), and mixed variety (EMX) swirl cultures.

| | Methanol | Ethanal | Ethanol | 1-propanal/ 2-propanone RT=7.1 min | C-4 pks | 1-butanol | RT=12.7 min | 3-hydroxy- 2-butanone RT=17.8 min |
|---|---|---|---|---|---|---|---|---|
| ECA | 1.3 a* | T a | 69.9 a | 1.1 b | 6.6 a | 19.9 a | T a | T b | T a | T b |
| ECC | 0.7 a | T a | 82.1 a | 1.0 b | 1.3 a | T b | T a | T b | T a | T b |
| EMX | 2.3 a | T a | 83.6 a | 1.0 b | T a | 1.6 b | 1.4 a | T b | T a | T b |
| CO | T a | T a | 2.4 b | 64.4 a | T a | T b | T a | 14.6 a | T a | 9.6 a |

1)Percentage of total peak areas.

*Values within columns followed by the same letter are not significantly (P = 0.05) different by Duncan's test for ordered means.

T = Trace.

## SUMMARY OF RESULTS AND DISCUSSION

Variability in the rate of disease development was a consistent problem in the jar experiments. The infections appeared to catch at different rates in the various replicates. Once the disease had become established at one point on the tuber, general decay was rapid due to the spread of the bacterial seepage over the tuber surface. Generally the E. carotovora decay became visible by day 5 to 9 of the incubation period. After 13 to 15 days, the tubers were almost completely decayed.

Contamination of the control treatments with E. carotovora bacteria was also a problem in the jar experiments. Whether the infections arose from bacteria not eliminated in the sterilization process or were due to some post-sterilization contamination could not be determined. The manufacturers specifications, as well as preliminary tests indicated that the described sterilization procedure was effective in eliminating surface contaminants. However, E. carotovora bacteria could have been located in the lenticels or cracks in the tuber surface. These locations may not have been penetrated by the Teramine. Although both varieties of E. carotovora were isolated from the contaminated controls, the variety specific inoculated treatments did not show any crossover contamination. This would seem to indicate that the E. carotovora bacteria were introduced as contaminants after the sterilization process.

Staphylococcus sp., Clostridium and various yeasts dominated the secondary microflora isolated from the bacterial seepage of the disease treatments. Population levels of the secondary contaminants were low in comparison with the E. carotovora levels. Very few contaminants were isolated from the

healthy controls.

The source of the contaminant organisms was uncertain. It is possible they arose from populations sheltered within surface cracks, or present in the tubers' vascular system (Hayward 1974).

Although the populations of secondary microflora were relatively small, their potential contributions to the volatile profiles should not be ignored. For instance, the yeast could be expected to produce a wide range of short-chain alcohols. These compounds were important components of the observed profiles.

Harrison and Nielsen (1978) reported that pure E. carotovora infections of potatoes had only a mild odor. Only when the normal secondary microflora was allowed to develop did the decay take on the unmistakable odor normally associated with rotting potatoes. However, the perceived strength of an odor is not necessarily a reliable indication of the concentration of all the compounds in a headspace sample. Some of the compounds isolated in the potatoes' volatile profiles are almost odorless (i.e. N-butanol).

In the bag experiments, signs of decay were apparent by day 2 of the incubation period. By day 4 to 5, the accumulation of bacterial seepage prevented the further sampling of the bags. By that point, the test tubers were totally disrupted.

Tuber decay was much more rapid in the bag than in the jar trials. It is possible that the direct contact between the tubers and the plastic bag produced the localized anaerobic conditions vital to the E. carotovora development (Perembelon and Kelman 1980). There was also the potential for the exchange of the pathogen from tuber to tuber within the bags. This exchange would tend to produce a rapid developing, relatively uniform infection.

In the bag experiments, the microflora community and E. carotovora populations isolated from the bacterial seepage were very similar to those observed in the jar experiments. The contaminant populations tended to be lower than in the jar experiments, possibly due to the shorter period of substrate availability and population increase. Consequently, the significance of the secondary contaminants' contributions to the volatile spectrum should have been reduced relative to the situation in the jar experiments.

E. carotovora and Control Volatile Profiles

In both the bag and jar experiments, there was a dramatic increase in the total volatile production of the inoculated treatments as the E. carotovora infection developed. In the jar experiments, total volatile production increased by 36 to 280X over the 13 to 16 day test periods. In the bag experiments, production levels at the end of the 4 to 5 day incubation period ranged from 2X (Experiment 5) to 66X (Experiment 4) the output at the beginning of the tests.

In the jar experiments, elevated volatile outputs were consistently detectable within 3 to 5 days of the introduction of the pathogen. In the bag experiments, the elevated volatile outputs were detectable within 24 h of the injection.

The volatile metabolites produced in any given disease infection will involve a combination of compounds produced by the host, by the pathogen, and by the unique physiological processes involved in the host/pathogen interactions (Richard-Moulard et al. 1976; Cole 1980; Abramson et al. 1980).

During the development of an E. carotovora infection in potatoes, volatile production by the host tissues increases due to the increased metabolic activities in the wounding response and/or any pathogen resistance processes (Varns and Glynn 1979). Volatile production by the pathogen is

dependent upon the type and availability of substrate, growth conditions, and most importantly, the pathogen population (Henis et al. 1966; Richard-Moulard 1976).

In both the jar and bag experiments, the rate of increase in the total volatile production from day to day was greater towards the end of the test period. Except in cases where experimental variability was excessive, the total volatile output data in both the jar and bag experiments fit exponential equations for increase over the test periods.

Although pathogen population levels were not monitored during the tests, under favorable conditions pathogen populations could be expected to increase exponentially (Stevens 1974). It appears that the exponential pattern of increase in the total volatile output could be a direct reflection of the metabolic processes of the growing pathogen population. Alternatively, the expanding pathogen population could have been triggering a parallel increase in the resistance activities of the host, which would again lead to an exponential type of increase in volatile metabolite production. In the bag trials, the Y intercept (incubation period = 0 hours) total volatile production values tended to be much greater than in the jar experiments. This would be expected as the volatile production from a larger mass of potatoes was being introduced as a concentrated sample into the gas chromatograph. The slopes for the regression lines also tended to be steeper indicating a more rapid disease development in the bag experiment.

In all experiments, chromatogram complexity (number of peaks/chromatogram) increased somewhat as the infections progressed. This was likely due to an increase in the detectability of the various compounds as the total volatile production increased. Changes in the metabolic processes of the pathogen with changes in the quality or availability of substrate could have led to

the production of additional metabolites, Further, the developing secondary microflora populations may have made some unique contributions to the volatile profiles as the incubation period progressed.

In the jar experiments, significant quantities of 13 compounds (four unidentified) were detected in the volatile profiles of the E. carotovora infected treatments (REFERENCE TABLE A), while 14 compounds (REFERENCE TABLE C) (five unidentified) were detected in the bag experiments.

All of the compounds that were tentatively identified in the jar experiments were subsequently isolated in the bag trials. It was not possible to verify that the various unidentified compounds in the respective sets of experiments were similar. However, comparisons of the retention times of the unknowns with the identity-verified compounds indicated some similarities between the two sets of experiments; i.e., an unknown peak occurred between the 1-propanal/2-propanone and 1-propanol peaks in both trials.

The compounds identified ranged from $C_2$ to $C_4$ and covered a number of functional groupings, i.e., alcohols, aldehydes, ketones. A number of the chromatogram components identified in this study were also isolated by Varns and Glynn (1979) in their work on the volatile profile of E. carotovora var. atroseptica; i.e., 3-hydroxy-2-butanone, ethanal, 1-propanol, ethanol, 2-propanone, 1-propanol, 2-methyl-1-propanol, and 2-butanone. A number of compounds identified in this study were not reported by Varns and Glynn (1979); i.e., methanol, 1-propanal, 1-butanol, and 2-butanol. Varns and Glynn (1979) also isolated a number of compounds which could not be detected in this study: i.e., butane, methyl and ethyl acetate, hydrogen sulphide, methyl sulfide, methyl disulfide, and propanethiol.

In light of Harrison's and Nielsen's (1978) finding that pure E. carotovora

infections were relatively odorless, it might be suspected that the highly odiferous sulphur compounds detected in Varns and Glynn's study may have been produced by secondary saprophytic microflora.

In general, the production levels of the individual volatiles increased as the infection developed. However, the responses of the various compounds were not identical. For example, in the bag experiments the output of the unidentified compound with a RT of 16.3 min increased very little over the incubation period, while the ethanol levels increased by as much as 2000X over the test periods. The same variability in compound production was observed in the jar experiments.

Chromatogram signatures, which involved the arrangement of the major chromatogram peaks in order of their average importance to the total volatile output were developed to facilitate the comparison of complex volatile profiles obtained with different experimental treatments and replicates. In the jar experiments, although the signatures developed for the diseased treatments were not identical between experiments, they were similar in terms of the relative importance and groupings of the various metabolites.

1-Propanol/2-propanone, ethanol, 1-propanol, 1-butanol, and 1-butanol/hyde/ 2-butanol were the dominant compounds, in terms of their peak area values, in the E. carotovora profiles. Although the dominant compounds did not occur in exactly the same order in the three jar experiments, they were always contained within one or two homologous groupings. Consequently, the differences in the actual order of the compounds were not likely overly important.

In general, the peak area and PTPA signatures were much less uniform in the bag trials than in the jar experiments. Ethanol, the C-4 compounds, 1-butanol, methanol, ethanal, and compound D dominated the volatile

profiles of the E. carotovora inoculated treatments. However, the importance of these compounds relative to each other changed considerably from experiment to experiment. The reason for this observed variability in the volatile profiles is open to question.

In the bag experiments, disease development was extremely rapid resulting in the very short incubation period. Consequently, the chromatogram signatures were based on a limited number of data points. Given that the importance of the various peaks relative to each other changed during the development of the infection (Figure 4a), it could be expected that any variability in the rate of disease development between the experimental treatments or replicates would be reflected in significantly different chromatogram signatures. In the jar experiments, any variability in the disease development would be averaged over a much greater number of data points, due to the extended incubation period. Consequently, the chromatogram signatures could be expected to be more stable. As previously discussed, variability due to the contributions of the secondary microflora should have been of relatively minor significance. Variability due to erratic trap or G.C. performances was not considered a problem.

There was some overlap in the chromatogram signatures prepared for the E. carotovora infected treatments in the jar and bag trials. Ethanol, 1-butanol, and the C-4 compounds were amongst the more important compounds in both sets of signatures. The variability in the relative importance of the remaining peaks reflected the considerable differences in the rate and patterns of disease development in the two series of experiments.

In both the jar and bag experiments, the PTPA signatures closely mirrored the peak area signatures in the order of importance of the various compounds. However, the PTPA signatures tended to have fewer statistically significant peak groupings than in the peak area signatures. The system utilized for

calculating the PTPA values appeared to introduce a measure of variability which interfered with the separation of the peaks in terms of their relative importance to the overall profile.

Volatile Production Characteristics of Control Treatments

The volatile production characteristics of the control treatments were considerably different from the disease treatment profiles. In comparison to the disease treatments, the daily volatile production of the controls remained very low and relatively stable over the incubation period. In the jar experiments, the average total volatile output of the E. carotovora infected treatments was 100X (Experiment 2) to 800X (Experiment 1) greater than for the controls. In the bag experiments, the diseased treatment average volatile outputs ranged from 2 to 200X that of the controls, depending upon experimental conditions. When comparisons were made solely on the basis of the production values at the end of the incubation period, the diseased treatment volatile outputs were often $10^3$X greater than the controls.

In the jar experiments, the control tubers produced a burst of volatile production at the very beginning of the incubation period. Varns and Glynn (1979) concluded that this volatile production was a non-specific host response to wounding (i.e., the damage produced by the sterile needle).

In both the jar and bag trials, the volatile production of the controls tended to increase somewhat over the incubation period. In the bag experiments, this increase in volatile production generally overshadowed any 'wounding' effect.

In the jar experiments, the natural logs of the daily volatile production data for the control treatments showed a statistically significant fit to the best-fit exponential regression lines. In the bag experiments, the quality of fit varied due to the limited number of data points involved. In

all the jar experiments, both the slope and the general regression equations for the control treatments were significantly different from those derived for the E. carotovora infected treatments. In the experiments where the data showed a significant fit to the regression equations, the slopes and regression equations for the E. carotovora infected and control treatments tended to be significantly different.

In both series of experiments, the regression equations reflected a number of differences in the patterns of volatile production. In most cases, the Y intercept was greater in the equations derived for the diseased treatments. This indicated that even with the increase in volatile production due to control wounding, the diseased treatments tended to exhibit significantly elevated volatile production levels very early in the incubation period. The regression equation slopes tended to be significantly steeper in the diseased treatments. This reflected the more rapid rate of increase in volatile production/unit time that occurred in the diseased treatments. This trend was more apparent in the bag experiments due to the more rapid disease development in these trials.

A total of eight compounds (all identified) were detected in the volatile profiles of the control treatments in the jar experiments (REFERENCE TABLE A). In the bag experiments, 12 compounds (three unidentified) were detected in significant quantities in the various control treatments (REFERENCE TABLE C). In both the bag and jar experiments, all of the identity-verified compounds in the control profiles were also present in the E. carotovora disease profiles.

Combining the data from the two experimental series, the only compounds that were found in the diseased treatments but not in the controls were a number of unidentified peaks (RT = 8.2, 16.0, and 18.5 min in the jar experiments; 14.1 and 16.3 min in the bag experiments). These compounds tended to be relatively unimportant in the volatile profiles of the disease

treatments (PTPA <1%). Consequently, the potential for utilizing the presence of these compounds to distinguish between diseased and healthy profiles would appear to be limited. Further, it should be stressed that the detection of any given compound is almost entirely dependent upon the sensitivity of the detection apparatus. The number of peaks/chromatogram for the control treatments tended to increase over the incubation period. This again could have been due to changes in the metabolic patterns over the test period, but more likely was due to an increase in the detectability of the various compounds as the total volatile production increased.

On a day to day basis, the control chromatograms were consistently less complex than in the diseased treatments. In all of the jar experiments, and most of the bag trials, the mean number of peaks/chromatogram, averaged over the entire incubation period, in the control profiles, was significantly lower than for the infected treatments. Even though there were few compounds present in the diseased profiles which were not at one point or another detected in one of the controls, the control profiles could consistently be differentiated from equivalent diseased profiles on the basis of their relative complexity.

In the jar experiments, the signatures for the controls were not identical, however, there were a number of similarities in terms of the relative importance and groupings of the various metabolites. 1-Propanal/2-propanone, ethanol, 1-propanol, 1-butanol, and 1-butanal/2-butanol were the dominant compounds in terms of their peak area values. As in the E. carotovora signatures, the exact order of these compounds in the control signatures was not stable. Again, the dominant compounds tended to be included within a single homologous grouping in the signature, indicating that the precise rank of the compounds was not overly significant.

In the bag experiments, the peak area and PTPA signatures for the control treatments were again much less stable than those for the jar experiments. There was little consistency in terms of the compounds that dominated the signatures. In most cases, the majority of the compounds in the signatures were included within one or two statistically homologous groupings, indicating that the production levels of the various compounds was relatively uniform. Any changes in volatile production over the incubation period must have been relatively non-specific, which agrees with Varns and Glynn's (1979) data on the non-specificity of the wounding response of healthy tubers.

In both the jar and bag experiments, the PTPA signatures for the controls closely mirrored the peak area signatures in terms of the order of importance of the various compounds. Again the PTPA signatures tended to have fewer statistically significant peak groupings than in the peak area signatures.

The chromatogram signatures developed for the control and diseased treatments in the jar experiments were relatively similar. They contained a similar range of compounds and the same group of compounds (1-propanal/2-propanone, ethanol, 1-propanol, 1-butanol, and 1-butanal/2-butanol) were dominant in both sets of signatures. In each of the jar experiments, when the average peak areas ($LOG_{10}$) for the various chromatogram components were compared, a number of significant differences were found between the profiles of the E. carotovora infected and control treatments (Tables 1g and 2i). In all cases, the diagnostic difference between the profiles was due to volatile outputs of the diseased treatment being significantly greater than those of the controls. However, in the two jar experiments, there was no single compound which, in both trials, occurred at significantly different levels in the diseased and control profiles.

Comparisons between the signatures for the diseased and control treatments in the bag experiments were complicated by the variability of the signatures. The range of compounds involved in the signatures was similar, but a dominant group of compounds in the control signatures could not be isolated.

In comparing the mean peak area ($LOG_{10}$) values for the various metabolites, significant differences were again discovered between the diseased and control profiles in each of the different bag experiments. Again, in almost all cases, the diagnostic differences were due to the significantly greater volatile production by the diseased treatments. In comparing the results for the various experiments, it again became apparent that there was no individual or consistent group of compounds which occurred in significantly different quantities in all of the diseased and control profiles.

The cause of this inconsistency in both the jar and bag experiments was excessive experimental variability introduced by the uneven development of the diseased treatments and the erratic volatile production of the controls. There were many cases where the average production level of a component in the diseased treatment profile was 100X greater than in the controls, but the difference was not statistically significant.

Excessive experimental variability also obscured any consistent differences between the PTPA values for the individual compounds in the control and diseased treatment profiles.

Wounded and Non-Wounded Control Comparisons

Any surface damage or penetration of potatoes triggers the tubers' localized disease resistance and wound-healing mechanisms (Lichtovich et al. 1967). The increases in metabolic activity linked to these defense responses are reflected by an increase in the volatile output of the damaged tissues (Varns and Glynn 1979).

In all the experiments conducted in this study, the process of introducing the selected pathogenic bacteria entailed some wounding of the tubers. In order to determine the tuber response to wounding, all control treatments were wounded in an identical manner. Interestingly, in many cases the wounded control treatments showed very significant volatile production levels. In some cases, the volatile production levels were similar to those of the inoculated treatments (Table 1a). The volatile production levels of the controls tended to increase over the incubation period (Table 1a; Figure 4b). These results do not agree with the concept of the wounded response as a short-term (1 to 2 day) reaction which peaks immediately after the damage occurs (Lichtovich et al. 1967; Varns and Glynn 1979).

In two experimental trials 'healthy', non-wounded controls were monitored in an attempt to determine whether the wounding effect alone was responsible for the previously observed volatile production patterns of the wounded control (CO) treatments. Given that the non-wounded potatoes were being maintained in a uniform suitable environment, it was expected that their metabolism and therefore their volatile output patterns would remain relatively stable over the incubation period.

Unfortunately, contamination of several replicates of both the wounded and non-wounded treatments sharply limited the validity of any comparison that could be made between the treatments. In general, the total

volatile production of the non-wounded controls was significantly lower than for the wounded treatment (Figure 5a) which is consistent with the concept of wound-induced volatile production. However, as in the previous observations of the wounded controls, the total volatile production of the non-wounded controls increased steadily over the incubation period. This would seem to reduce the likelihood that the inoculation-related wounding was the root of the escalating volatile production of the wounded controls. The cause of the increasing volatile production by the wounded and non-wounded controls is uncertain. It is possible that the volatile build-up could have been indicative of the growth of contaminant microbe populations even though no significant populations were recovered. Alternatively, if the incubation environments were in some respect unsuitable for the tubers, the resulting stress reactions could have led to the increased volatile production (Varns and Glynn 1979).

The relationship between wounding and volatile production, as well as the volatile production characteristics of 'healthy' tubers require more thorough investigation.

Comparison of E. carotovora var. carotovora and E. carotovora var. atroseptica

The division of the E. carotovora species into a number of varieties was based on the results for a number of very specific biochemical tests (Dye 1968; Graham 1972; De Boer and Kelman 1975). The E. carotovora varieties also show different temperature tolerances.

In terms of most aspects of their physiology and physiochemistry, ECC and ECA are extremely similar. The primary metabolic pathways and associated enzymes systems are identical (Graham 1964; Starr and Chatterjee 1972). The histopathology and pathogenic characteristics of the varieties are essentially indistinguishable (Graham 1964; Perembelon and Kelman 1980). Both varieties are common pathogens in potato storages. In order to allow for the comparison of the volatile profiles of the E. carotovora varieties, metabolite monitoring tests were conducted using an incubation temperature that was in the optimal growth range of both ECC and ECA ($22^{o}$ C).

In the two jar experiments, the E. carotovora varieties performed relatively consistently, yielding stable volatile profiles. There were no appreciable differences in the overall volatile production patterns of the two varieties. The rate of increase and the scale of the total volatile outputs/unit time were relatively similar for ECC and ECA (Figures 2b and 3a).

Three of the bag experiments involved comparisons between the E. carotovora varieties. In Experiment 6, different strains of the varieties were tested and the data for the different strains for each variety were combined to allow for a comparison between the varieties. In Experiment 8, the varieties were tested at different incubation temperatures, only the data from the $22^{o}$ C trial was considered in this analysis, as the temperature effect was significant. In the individual bag experiments, the

volatile production characteristics of the E. carotovora varieties were often significantly different. However, these differences were not consistent; completely opposite reactions often occurred within replicates of a single experiment. Extreme, yet inconsistent variability in the rate of development of the E. carotovora varieties in the different experiments made comparisons between the varieties very difficult.

In both the jar experiments, the total daily volatile outputs of both E. carotovora varieties increased exponentially over the duration of the incubation period. Covariant analyses did not produce any conclusive evidence for differentiation of the E. carotovora varieties on the basis of either the slope or the general equations for the volatile production responses (Tables 2f and 3d).

In the bag experiments, the daily volatile production data for the different E. carotovora varieties usually showed a significant fit to the exponential regression equations (Tables 5a, 6e, and 8a). The results for the covariant analyses of the regression lines for the E. carotovora varieties were erratic. The results in each experiment reflected the variability in the rates of disease development.

In both the jar and bag experiments, the disease varieties could not be differentiated on the basis of the daily volatile production levels, nor were there any consistent differences in the average total volatile output levels of the ECA and ECC treatments (Tables 2c, 5c, 6k, and 8c).

In both the jar and bag experiments, all recorded metabolites were common to the profiles of both disease varieties (REFERENCE TABLES A and C), although differences were noted in the chromatogram complexity (number of peaks/chromatogram). These differences were not consistent enough to justify differentiation of the profiles of the E. carotovora varieties.

The metabolite signatures for each of the E. carotovora varieties were not consistent in the two jar experiments. Different compounds dominated the signatures in each case, although in both experiments the primary components tended to be included within the first or second homologous peak groupings.

The variety signatures appeared to more closely resemble the signature of the other E. carotovora variety in the same experiment more than they resembled the signature of the same variety in a different experiment (Tables 2h and 3f). It would appear that the variability from experiment to experiment was more significant than any differences between the disease varieties.

In the bag experiments, the peak area signatures developed for the E. carotovora varieties were even less stable than those developed in the jar experiments. There was very little consistency in compounds that dominated the signatures. The signatures continued to be divided into a number of distinct homologous groupings, indicating that the lack of a consistent group of dominant compounds was not due to uniform production levels for the various compounds.

In both the jar and bag experiments, the PTPA signatures tended to be somewhat more stable than the peak area signatures. In the jar signatures for both disease varieties, 1-propanol/2-propanone and ethanol were the dominant peaks in terms of their PTPA values. In the bag experiments, methanol and ethanol were consistently within the dominant group of peaks in the PTPA signatures of both E. carotovora varieties. From these data, it would appear that the system utilized in calculating the PTPA values was effective in avoiding some of experimental variability encountered in the peak area signatures.

All comparisons of the signatures for the varieties were complicated

by the variability within the signatures for each variety. In comparing the mean peak area ($LOG_{10}$) values for the various metabolites in the jar experiments, a number of statistically significant differences were found between the profiles of the E. carotovora varieties (Table 2i and 3g). However, there were no diagnostic metabolites common to the two experiments, each yielded a completely unique range of diagnostic characteristics. Further, there was no consistency in terms of which of the E. carotovora varieties produced the significantly greater amount of the diagnostic metabolites.

In the bag trials, there were relatively few significant differences between the peak area values for the major metabolites in the profiles of the E. carotovora varieties (Tables 5g, 6n, and 8i). Again, there was no individual or group of compounds which could have been used to differentiate between disease varieties in all of the bag experiments.

There were no consistent differences in the PTPA values of the various metabolites which could have been used to differentiate between the ECA and ECC profiles in either of jar and bag experiments.

In summary, in both the jar and bag experiments, no consistent differences were apparent in the volatile profiles of the E. carotovora varieties. Whether this indicated that the varieties were similar in terms of their volatile production characteristics under these test conditions remained uncertain due to the excessive variability encountered in all of the experiments.

Differentiation Between Strains Within E. carotovora Varieties

In previous experiments, the performance of the E. carotovora varieties had been erratic. In theory, this variability could have been linked to the viability/pathogenicity of the test strains of E. carotovora. These experiments were designed to determine whether the disease responses varied with different strains of bacteria. The previously utilized strains of ECA and ECC were compared against various additional isolates.

The performance of the various strains in the two trials was extremely erratic. The relationships between the volatile profiles of the various strains of each E. carotovora variety were not stable.

In both trials, ECA1 consistently produced greater total volatile outputs than ECA2, but the difference was significant in only one trial (Table 6c). There were no consistent differences in the total volatile outputs of the three ECC strains.

The covariant analyses of the regression equations developed in Trial 1 indicated that there were significant differences between the rates of volatile production by the various strains (Table 6c). However, in light of the observed variability in the performance of the strains, the validity of any comparisons based upon a single experimental trial would be questionable.

In both trials, all major chromatogram peaks were common to the volatile profiles of the various strains. However, the ECA1 chromatograms were consistently more complex than those for ECA2. The ECC strains did not show any consistent differences in chromatogram complexity (Table 61).

The chromatogram signatures for the strains of each E. carotovora variety were relatively similar in terms of the dominant compounds and the peak groupings. Again the signatures for the different strains in

each trial tended to resemble each other more than the signature for each strain matched the signature obtained in the other trial. Comparisons of the peak area and PTPA values did not indicate any significant differences between the profiles of the various strains tested for each E. carotovora variety.

When the data from the respective strains for each E. carotovora variety were pooled, the volatile production characteristics of the E. carotovora varieties were similar to those observed in previous experiments.

In natural populations of any pathogen, genetic variability in the population leads to diversity in the relative pathogenicity of the various strains of the pathogen (Day 1978). In laboratory cultures, selection pressures not normally encountered in natural conditions may lead to the development of aberrant or attenuated populations (Stanier et al. 1976). Consequently, the utilization of several distinct pathogenicity-verified strains is considered desirable in any pathogenicity study. This would be particularly important in analysis of any soft rot infection since the rate and degree of host decay could, in theory, be strongly influenced by the viability and relative pathogenicity of the E. carotovora strain (Poff 1979; Perembelon and Kelman 1980).

In summary, the various strains of ECC tested appeared to have very similar disease characteristics and volatile production profiles. The ECA strains could be differentiated on the basis of their total volatile production characteristics but comparisons involving individual compounds did not indicate any consistent differences. The differences in total volatile production appeared to reflect differences in the rate of disease development in the two ECA strains.

In all experimental trials, the experiment to experiment variability

had a more significant impact on the volatile profiles than any differences between the various strains of each E. carotovora variety.

Incubation Temperature Effects

Temperature could be expected to have a very significant impact upon the volatile production and diffusion characteristics of both healthy and E. carotovora infected potatoes. The growth of E. carotovora ceases below 4° C, reaches a maximum at about 25 to 30° C (Q10 $\simeq$ 2), and ceases again above 37 to 39° C depending upon the E. carotovora variety involved (Perembelon and Kelman 1980). The wound-healing and disease resistance reactions of the potato are also temperature dependent. Commercial storage temperatures are elevated to around 15° C to promote maximum wound-healing in the new crop before the storage is cooled to the optimal longterm storage temperature (Nash 1978). Because the rate of metabolic activity in both the pathogen (E. carotovora) and the host are temperature dependent, it could also be expected that the rate of volatile metabolite production by the host/pathogen interaction would also be temperature dependent.

The rate at which a metabolic volatile is released from a biological system is dependent upon the rate of diffusion from the compound's point of origin. Diffusivity is, in turn, a temperature dependent phenomenom (Kolb 1976a, b). Further, the activity or volatility of the various metabolites would also increase as the system temperature increased. This would also tend to increase the rate of volatile release from the tuber tissues.

In previous experiments, the tubers had been incubated at 22° C. Clearly, the volatile production and diffusion characteristics at this temperature would be expected to differ from the situation at normal storage temperatures (8 to 12° C).

The rate of development of the visual symptomology (i.e. bacteria seepage and tuber collapse) in the inoculated treatment was clearly

temperature dependent. At 22° C, decay was complete within 5 days, at 9.8° C the destruction was somewhat slower, and at 4.1° C, the infections were still restricted to the inoculation site at the end of the incubation period.

In general, the total volatile outputs of the diseased and control treatments exhibited the expected increase with increases in the incubation temperature. In some cases, the average volatile outputs of the high temperature control treatments were significantly greater than those of the diseased treatments being held at a lower temperature (Tables 7a and 8c). Only the difference in volatile production between the 22 and 4.1° C incubation temperatures was significant (Table 7e), but in the second experiment the average peak areas of the 22 and 9.8° C treatments were also significantly different (Table 8c). In most cases, the diseased and control treatments were affected to a similar degree by the changes in the incubation temperature (DXT interactions non-significant), but the ECC and ECA treatments responded differently, as will be discussed in a subsequent section.

The regression equations for the daily total volatile output also reflected the importance of the incubation temperature. The Y intercepts for each treatment at the higher incubation temperature tended to be greater than at the lower temperature (Tables 7b and 8a). This greater initial volatile production may have been related to the more pronounced wounding response at the higher temperatures. In the first experiment, the slopes of the regression equations for the diseased treatment became progressively steeper as the incubation temperature was increased. The rate of disease development and consequently the volatile output were clearly strongly influenced by the incubation temperature. The same trends were apparent, but less distinct, in the second experiment.

Altering the incubation temperature did not lead to any differences

in the range of volatiles detected for each treatment. Despite the expected decrease in the production levels of the individual compounds at the lower temperatures, concentrations were sufficient to allow for detection of the compounds. However, the mean number of peaks detected in the daily chromatograms was strongly influenced by the incubation temperature. For all treatments, each increment in the incubation temperature resulted in a significantly greater chromatogram complexity than the next lower temperature (Tables 7g and 8f). Again, it was not unusual for control treatments at a higher temperature to have a greater average number of peaks/chromatogram than diseased treatments at a lower temperature.

Changes in the incubation temperature could potentially alter the signatures for the individual metabolites in one of two ways; 1) if the changes in the production of the individual metabolites are non-specific and relatively equivalent, the peak area and PTPA signatures at the various temperatures could be expected to be relatively similar; 2) if the changes were not equivalent across the volatile spectrum, the result would be significantly different peak area and PTPA signatures at the various temperatures. The latter seems the more likely pattern. In previous experiments, it had been found that the volatile production pattern (amounts and ratios of compounds) was not consistent over the duration of the incubation period. Consequently, if changes in the incubation temperature produced inequalities in the rate of disease development or wound healing, these inequalities would be reflected as variability in the metabolite signatures. Additionally, the vapor pressures of the individual compounds would respond differently to any given temperature change (Appendix A ). In theory, the differential response should lead to changes in the relative importance of the various metabolites.

In comparing the peak areas or PTPA values of individual metabolites

across incubation temperatures, it would be expected that if the changes in volatile output with temperature were non-specific there would be consistent differences in peak areas (high > low temperature), but the PTPA values for the individual metabolites should remain relatively stable. However, if the changes in the volatile spectrum with temperature were non-equivalent then significant differences in both the peak area and PTPA values could be expected. It was difficult to make comparisons between the peak area and PTPA signatures for each treatment in the first experiment. The peak rank was not identical at the various temperatures but the same group of dominant compounds always occurred within the first or second homologous groupings (Table 7i). In the second experiment, the results of the signature comparisons were inconsistent in the two trials (Table 8m). In the first trial, both the peak area and PTPA signatures varied considerably from the 22° C to the 9.8° C incubation temperatures. This would seem to indicate a non-equivalent volatile profile change with temperature changes. However, in the second trial the signatures at the two incubation temperatures were very similar. This would seem to indicate an equivalent change in volatile production with changing temperature.

In the first experiment, there were very few individual peaks with significantly different output levels at the various temperatures (Table 7l). It would seem likely that the expected differences in the peak areas were not detected due to excessive variability, since the total peak areas consistently indicated differences between the various incubation temperature treatments. In both trials of the second experiment, the peak areas for a number of compounds were significantly different at the two incubation temperatures (Table 8n). As was expected, in all cases the differences were due to the greater outputs at the higher temperature. There were also

a number of compounds that exhibited significant shifts in their relative importance (PTPA value) as the incubation temperature changed. However, the two trials showed no consistent changes; most compounds were found to have changed significantly in only one of the trials. In some cases, a compound became more significant as the incubation-temperature increased in one trial and less significant in the other. It would appear that very few conclusions as to the potential effect of the incubation temperature on the volatile profiles could be drawn from the data for the individual peaks. Variability was excessive in all trials.

In summary, the incubation temperature had a significant impact on the volatile profiles recorded for both E. carotovora infected and healthy tubers. This impact was related in part to temperature dependent changes in the metabolic activity involved in the host/pathogen interaction. Additionally, the potentially significant physical processes of metabolite volatilization and diffusion were also sensitive to temperature changes.

The combined impact of these two factors affected both diseased and healthy treatments. Total volatile production for any given treatment could be expected to increase as the temperature increased. At lower temperatures, the differences between diseased and control profiles became progressive less distinct. The short incubation periods utilized in these experiments were not sufficient to allow for the detection and monitoring of the very gradual changes in metabolic activity and volatile output that are characteristic of slow developing infections.

The patterns of production of the individual components of the volatile profiles could also be expected to change with the incubation temperature. This factor could become significant when comparisons are made between profiles obtained under different conditions.

Clearly, the potential impact of temperature must be seriously considered prior to the utilization of volatile monitoring as a technique for the detection of disease development in commercial storages.

Incubation Temperature X E. carotovora Variety Effects

The relative rates of development of E. carotovora var. atroseptica and E. carotovora var. carotovora are temperature dependent (Perembelon and Kelman 1980). In order to ascertain the impact of temperature-induced differences in the rate of disease development on the volatile outputs of the E. carotovora varieties, tests were conducted at temperatures that promote both equal and differential growth of the E. carotovora varieties.

The ECC and ECA treatments did not show any differences in their visual symptomologies at the two incubation temperatures (22 and 9.8° C). Averaged over both incubation temperatures, the mean total volatile outputs for the ECC and ECA treatments were similar. However, in both trials there was a significant disease treatment X incubation temperature interaction. At 9.8° C, the ECA treatment consistently produced greater total volatile outputs than ECC, while at 22° C, the ECC outputs were consistently greater than ECA (Table 8c). Although the differences were not always statistically significant, they agreed with the expected patterns. At 9.8° C, the ECA infections would have been expected to develop more rapidly than ECC (Perembelon and Kelman 1980). Although 22° C is in the optimal range of both varieties, it is possible that differences in isolate performances could have produced the consistent differences between the varieties.

The volatile production regression equations for the ECA and ECC treatments at the two incubation temperatures were not consistently different. However, visual inspection of the lines indicated the existence of variety specific differences in the response to the incubation temperature. At 22° C, the daily volatile production of ECC is consistently greater than for ECA, while at 9.8° C, the opposite was true (Figure 8a).

There were no temperature dependent differences in the range of compounds detected in the volatile profiles of the E. carotovora varieties.

However, at 9.8° C the ECA chromatograms were consistently more complex than those of ECC, due to more rapid development of the ECA infection at this temperature (Table 8f). At 22° C, the ECC and ECA chromatograms were equally complex.

The data for the individual peaks did not indicate any temperature dependent differences in variety performance. Given that the peak area and PTPA signatures for ECC and ECA were relatively similar at 22° C, it might be expected that at 9.8° C any difference in the relative progression of the ECC and ECA infections might be reflected in the peak areas and metabolite signatures. When making statistical comparisons between the individual metabolites, it might be expected that at 9.8° C the ECA profile should contain a number of compounds at significantly greater levels than in the ECC treatment. However, in both trials the signatures for ECC and ECA at 9.8° C were no more distinctive than at 22° C (Table 8m). Further, there were no more significant differences between the ECC and ECA profiles than at 22.0° C. Any differences were equally divided in terms of which of the E. carotovora varieties was producing the greater outputs. It would appear that the sensitivity of the individual peak area data to experimental variability obscured any temperature-specific differences in the performance of the E. carotovora varieties.

In summary, the disease development and volatile production patterns of ECC and ECA were relatively similar at 22° C. However, at 9.8° C, the more rapid development of the ECA treatment lead to significant differences in the volatile profile of the E. carotovora varieties. The extreme speed at which the infections developed when the incubation temperature was increased beyond 25° C made it impractical to utilize the higher temperatures which favor the development of ECC over ECA (Perembelon and Kelman

1980).

These results again illustrate the importance of the relationship between the rate and degree of disease development and the volatile production patterns. Disease organisms which had produced very similar disease profiles at one temperature produced different profiles at a temperature which resulted in differential growth of the pathogens being monitored. This interaction between the factors influencing pathogen development and the resulting volatile output may be of considerable significance in terms of the potential for utilizing volatile monitoring as a disease detection system in commercial storages.

Inoculum-Size Comparisons

In E. carotovora infections of potatoes, the importance of the size of the initial inoculum is dependent upon various environmental conditions. If storage conditions have led to an oxygen deficit at the infection site, very few bacteria are required to initiate an infection (De Boer and Kelman 1975). If, however, the tuber tissues have an adequate oxygen supply, decay will not occur unless the number of E. carotovora bacteria present at the infection site is sufficient to produce localized anaerobiosis (De Boer and Kelman 1975).

In our tests, the pre-inoculation soaking of the tubers and the post-inoculation mistings were designed to induce tuber anaerobiosis, thereby promoting the rapid and uniform development of the E. carotovora bacteria. However, disease development, as indicated by both visual inspection and the volatile production patterns, was erratic in many of the experimental trials. It would seem that the tuber treatments were not always effective in rendering the tuber immediately susceptible to infection. In some cases the disease took hold without an appreciable delay, whereas in other replicates, there was an initial lag followed by the normal rapid development of the infection. Based upon the assumption that the tuber tissues were not anaerobic, this initial delay may have represented the period necessary for the E. carotovora populations at the injection points to reduce local oxygen tensions to the decay thresholds.

It might be expected that variability in the size of the initial bacterial population at each infection point would be reflected in the length of this lag period. Experiments were designed to determine if variability and/or generally insufficient infection site bacteria populations were responsible for the erratic disease development and volatile

production patterns observed in previous experiments.

In both trials, visual observations did not indicate any significant differences in the rate of disease development between the two inoculum size treatments (SI = regular size and LI = 2X regular). Due to the experimental design used in both trials, the inoculum-size effects were confounded with the disease treatments and trap temperature effects. However, in all statistical analyses, the two- and three-way interactions between the inoculum size treatments and the other experimental variables were non-significant. Consequently, all comparison of the LI and SI treatments were based on the pooled disease treatment and trap-temperature data.

The volatile production patterns for the two inoculum sizes were relatively uniform. In both trials, the total volatile output averaged over the incubation period, for the LI treatment, was significantly greater than in the SI treatment (Table 9b). This would have been expected if the larger inoculum size had led to the more rapid development of the infection.

The total volatile outputs of both inoculum size treatments increased exponentially over the incubation period in Trial 1 (Table 9c). The slope of the regression line for the LI data was significantly steeper than for the SI data, indicating a more rapid increase in disease-induced volatile production. This is again consistent with the premise that a larger inoculum size could speed disease development. However, the overall regression equations were not significantly different. Additionally, the total volatile production at T = initial (Y intercept) was greater in the SI treatments (Table 9c). This represents a direct contradiction to the theory that disease development, and therefore elevated volatile outputs, should have been initiated earlier in the LI treatments.

The chromatogram profiles for the LI and SI treatments did not differ

significantly in terms of either the range of volatiles recorded or the average number of peaks/chromatogram (Table 9f). It might have been expected that had the LI treatment produced a more rapid decay, the average number of peaks/chromatogram should have been significantly larger than in the slower developing SI treatment.

The peak area and PTPA signatures for the inoculum-size treatments were not consistent in terms of the dominant peaks and peak groupings. This made any overall comparisons between the inoculum-size signatures difficult, but in general the LI and SI signatures in each trial looked to be relatively similar.

There were few significant differences between the individual peak area values in the LI and SI profiles (Table 91). It could have been expected that if disease development had been facilitated by the larger inoculum size, the peak areas of more of the profile components should have been significantly different (LI > SI). In both trials the PTPA values for all the major metabolites were indistinguishable; a further indication that the LI and SI infections did not develop in a significantly different manner.

In summary, the results from the investigation of the impact of the inoculum size upon the disease development and volatile production patterns were inconclusive. Several of the volatile production factors indicated that disease development was somewhat more rapid in the LI treatments, but various other profile parameters indicated that varying the inoculum size within the selected range had no significant impact on disease development or volatile production.

It should be noted that the E. carotovora populations at the end of incubation period were found to be roughly equivalent in the inoculated LI and SI treatments. This may have been an indication that the selected

inoculum sizes were not having a significant impact upon disease development. Alternatively, it is possible that the bacterial populations in the LI treatments may have reached the final observed population levels early in the incubation period and then may have stopped expanding due to limitations in substrate availability. This would seem possible since the potatoes were completely disrupted by the end of the incubation period. In retrospect, experiments involving lower inoculum dosages might have provided more conclusive information on the importance of inoculum-size as a determinant of disease development patterns.

Cultivar Comparisons

Self (1967) has published a review of the volatile metabolites that have been detected in potatoes. However, very little information is available on the volatile profiles of different potato cultivars. The majority of the previous research in this field has involved the testing of the volatile profile characteristics of different cultivars during various processing procedures. The only information of the volatile production characteristics of different cultivars in conditions suitable for the development of disease has come from Varns and Glynn's (1979) storage study. They found that bins of 'Kennebec' tubers infected with E. carotovora and Fusarium dry rot had significantly different volatile production profiles than similarly infected bins of 'Russet Burbank' tubers. Varns and Glynn (1979) concluded that the differences in the volatile profiles reflected differences in the rates of disease development in the two cultivars. This difference in the rate of disease development would be related to the relative suitability of the potato as a disease substrate versus any differences in the disease resistance capabilities of the cultivars.

'Norland' (table stock) and 'Russet Burbank' (processing) tubers were selected as the test cultivars in the experiments due to their prevalence in Manitoba's potato storages and because the cultivars are relatively different in terms of their dry matter content, etc.

The 'Norland' and 'Russet Burbank' tubers were compared in two jar experiments (Experiments 2 and 3) and in one replicate of one of the bag experiments (Experiment 6). In each of the experiments, the potato cultivar effects were confounded by the disease treatment effects. However, in all statistical analyses the potato cultivar X disease treatment interaction was non-significant. This indicated that any differences between the cultivars were constant relative to the disease treatments. Consequently,

the cultivar differences were analyzed as simple main plot effects.

In the jar experiments, considerable experimental variability existed in the disease responses of the 'Russet Burbank' and 'Norland' tubers. In one experiment there were no consistent differences in the total daily volatile outputs of the cultivars (Figure 2b). In the other experiments, disease development, as indicated by the rate of increase in the daily volatile production was considerably slower in the 'Russet Burbank' tubers (Figure 3a). In both experiments, there were no significant differences between the cultivars when the total volatile outputs were averaged over the entire incubation period.

In the bag experiments, the total daily volatile production of the 'Norland' treatments was consistently less than that of the 'Russet Burbank' treatments but averaged over the entire incubation period, the total volatile outputs of the potato cultivars were not significantly different (Appendix 6a).

In both the jar and bag experiments, all recorded chromatogram peaks were common to the profiles of both cultivars. At the various stages in the incubation periods, there were no consistent differences in the chromatogram complexities of the cultivars, nor were the average number of peaks/chromatogram significantly different (Appendix 2b, 3b, and 6b).

The chromatogram signatures developed for the cultivars in each of the jar experiments were not consistent. Again the signatures for the different cultivars within each experiment were more similar than the signatures for each cultivar in the different experiments. In Experiment 3, the peak area signatures for both cultivars involved only one or two homologous groupings, indicating that the peak areas for the various metabolites were either very similar or highly variable.

In only one of the jar experiments, could any significant differences be detected between the potato cultivars, in terms of the peak areas of any of the major metabolites. These differences were not consistent, in that one cultivar did not produce the greater amount of all of the diagnostic compounds.

In the bag experiment, the peak area signatures for the two cultivars were relatively similar. The same group of metabolites (1-butanol, the C-4 compounds, ethanol, and ethanal ) was dominant in both signatures (Table 6o). Although the exact rank of these compounds was not identical in both signatures, they were always all contained within a single statistically homologous peak grouping, indicating that any difference in the exact order of the compounds was not overly significant.

According to Henis' et al. (1966) system of analysis, there were several metabolites which could have been used to differentiate between the peak area signatures of the potato cultivars in the bag experiment. However, the lack of replication brings the validity of these differences into question. If the results for the jar and bag experiments are compared, there were no identity-known metabolites that were consistently considered to be diagnostic between the cultivars in terms of their peak area values. Although the last peak in the chromatograms of both the jar and bag experiments occurred in significantly greater quantities in the 'Russet Burbank' profiles, this could not be considered a reliable diagnostic feature as the peak identity could not be determined.

In both the jar and bag trials, the PTPA signatures closely paralleled the peak area signatures. No consistent diagnostic differences between the PTPA values for the cultivars were observed.

In theory, 'Norland' might be expected to provide a somewhat more

suitable medium for the initial development of E. carotovora bacteria due to its higher water content. It might be expected that the more rapid disease development in the 'Norland' tubers would be reflected in a more rapid increase in volatile production (Varns and Glynn 1979). The greater dry matter content of the 'Russet Burbank' tubers might indicate a potential for greater total volatile output due to the larger mass of useable substrate. However, no consistent differences were apparent in the volatile production patterns of the two cultivars. Further, the two cultivars showed similar disease reactions and volatile outputs when exposed to the various disease treatments. Whether this indicates that the volatile production characteristics of the cultivars were in actuality similar remains uncertain due to the excessive variability encountered in the very limited number of experimental trials.

Trap-Temperature Comparisons

The amount of any compound that may be retained without breakthrough on a given size porous polymer trap is determined by a large number of factors, viz., compound size, volatility, polarity relative to the absorbent, volatile concentration, sampling rate, trap surface area characteristics, etc. (Jennings et al. 1974; Wyllie et al. 1978). Trap temperature is also an important operating variable. The relative retention capacity of any compound on a trap decreases as its vapor pressure increases (Bertuccioli and Montedoro 1974). Cooling the trap decreases the vapor pressure of the absorbed compounds ( Bertsh et al. 1974; Mackay and Hussein 1978). Consequently, within the limits imposed by condensation problems, trapping efficiency increases as the temperature decreases.

In all the bag experiments, the trap temperature was reduced to $1^{o}$ C. This was considered merely a precautionary measure as preliminary tests utilizing in-line back-up traps had indicated the retention capacities of the primary traps had been adequate, regardless of the trapping temperature.

To further investigate the impact of temperature on the specific retention characteristics of the Chromosorb traps, experiments contrasting E. carotovora profiles with controls were conducted utilizing two trapping temperatures ($1^{o}$ and $22^{o}$ C). These experiments were designed to test the impact of trap temperature on the measurement of the various volatile production patterns that had been assessed in previous experiments. Due to the experimental design used in both trials, the trap temperature effects were confounded with the disease treatment and inoculum size effects. However, since the two- and three-way interactions between the trap temperature treatments and the other experimental variables were non-significant all comparisons of the $1^{o}$ and $22^{o}$ C trap-temperature treatments were based on the pooled disease treatment and inoculum size data.

In both experimental trials, the average total volatile production and the average number of peaks/chromatogram recovered from the 1° C traps were consistently greater than in the 22° C traps. Although these differences were not statistically significant, they would seem to indicate some consistent performance improvements at the lower temperatures.

Varying the trap temperature did not have any impact on the range of compounds detected in the volatile profiles. At 22° C, the absorbent capacities of the trap were sufficient to retain detectable quantities of all the major chromatogram peaks.

The peak area signatures in Trial 1 for the 1° and 22° C trap-temperature treatments were not overly similar in terms of the relative order of the dominant peaks (Table 9j). However, the homologous peak groupings were relatively similar. In Trial 2, the trap-temperature signatures for the peak areas were very similar in terms of peak order and groupings. In both trials, the PTPA signatures for the 1° and 22° C treatments appeared to be quite uniform.

There were very few significant differences between the average peak area and PTPA values of the various metabolites measured at the two trap-temperatures (Table 9m). These results were particularly significant, in that even the most volatile compounds trapped at 1° C were still trapped in statistically equivalent quantities when the traps were operated at room temperature.

In summary, trap performance at 22° C appeared to be comparable to the relative efficiency at 1° C. It should be stressed that this temperature-independent performance was based on a limited temperature range, and more importantly, a limited range of volatile compounds. Trapping efficiencies could be expected to change significantly if these experimental

parameters were altered (i.e. higher operating temperatures and/or more volatile compounds).

Volatile Profiles of Bacteria Cultures

The metabolic processes of the E. carotovora bacteria produced both quantitative and qualitative changes in the volatile profile of the swirl cultures. Since air exchange was allowed in the cultures, the observed increase in total volatile concentration was doubtless not an accurate representation of the actual changes in the volatile production of the inoculated cultures.

The ECA, ECC, and EMX treatments were very similar in terms of their total volatile production and also the range of metabolites detected in the headspace samples (Table 11a). This again reflects the similarity of the metabolic and developmental processes of the E. carotovora varieties. The PTPA values for the various metabolites in the inoculated cultures were, in many cases, significantly different from the PTPA values for the controls. This indicated that the increased volatile production in the inoculated treatments was not non-specific; certain volatiles exhibited proportionately different changes in concentration. The PTPA values of the three E. carotovora treatments were very similar.

The concentration of volatiles in the swirl culture headspace samples was minimal in comparison to the levels obtained with similar sampling procedures in the jar experiments. This would seem to indicate that the inoculum introduced in the various experiments was likely a relatively unimportant source of volatile contaminants.

All of the volatiles identified from both the inoculated and non-inoculated swirl cultures were subsequently isolated in the volatile profiles of the inoculated tubers. The parallel between the inoculated culture and the infection profile is not surprising since the metabolic processes of the E. carotovora bacteria could be expected to yield a similar

range of volatile by-products under both sets of conditions. The similarity between the volatile profiles of the non-inoculated cultures and the incubating potatoes may have been related to the fact that a potato infusion was used to prepare the potato dextrose swirl culture.

## E. carotovora and C. sepedonicum Comparisons

Bacterial ring rot presents an extremely serious threat to the crop in virtually every potato producing country (Knorr 1948; Shepard and Claflin 1975). In the field, Corynebacterium sepedonicum (Spieck and Knott) Skapt. and Burkh. infections lead to the stunting, wilting, and general decay of the growing plant (Manzer and Genereux 1978). In storage, contaminated tubers exhibit a characteristic internal breakdown of the vascular ring. During handling the infected tubers collapse spreading a highly infectious bacterial ooze over adjacent tubers. Although disease development is relatively slow under proper storage conditions, losses may be considerable (Shepard and Claflin 1975). As a seed-borne pathogen, ring rot outbreaks are a particularly serious problem during the storage of seed potatoes due to the potential for contamination of the entire seed stock.

In potato storages, prompt detection of any disease problem is a vital first step in the minimization of storage losses. However, correct identification of the specific disease problem is also extremely important. The recommended storage management procedures for disease control vary considerably from pathogen to pathogen. Consequently, the feasibility of utilizing volatile monitoring as a system for the detection of disease in storage would depend upon the selection of diagnostic volatiles or volatile production patterns that are as disease-specific as possible (Varns and Glynn 1979).

The goal of these experiments was to allow for the comparison of the volatile profiles and volatile production characteristics of C. sepedonicum and E. carotovora infections in order to assess the potential for utilizing volatile monitoring to differentiate between the infections in storage.

## C. sepedonicum Disease Summary and E. carotovora Comparisons

The rate and pattern of disease development in the C. sepedonicum

treatments was significantly different from that observed in the E. carotovora treatments. The first visual indication of decay in the C. sepedonicum infections became apparent by day 7 of the incubation period, while the first signs of decay were apparent much earlier in the E. carotovora treatments. In the C. sepedonicum infections, about 13 days were required before the tubers were completely disrupted by the pathogen.

As is typical in C. sepedonicum infections, seepage was minimal. By contrast the E. carotovora bacteria completely destroyed the tubers within 5 to 6 days, yielding copious amounts of bacterial seepage.

It was noted that the populations of secondary microflora were relatively large in the C. sepedonicum treatments. The extended disease development period in the C. sepedonicum infections may have allowed for the more extensive development of the secondary contaminants. In commercial storages, C. sepedonicum infections are commonly masked by the invasion of various secondary microorganisms, including E. carotovora (Manzer and Genereux 1978). The predominant secondary microflora in the C. sepedonicum infections (Staphylococcus, E. coli, and yeasts) were similar to those recovered from the E. carotovora infections.

Volatile Profile Comparisons

The volatile production characteristics of the C. sepedonicum treatments were considerably different from the patterns observed in the E. carotovora profiles. Many of these differences were related to the relative rates of disease development in the E. carotovora and C. sepedonicum infections.

The E. carotovora infection led to the production of elevated volatile outputs much more rapidly than the C. sepedonicum treatments (E. carotovora = 1 to 2 days; C. sepedonicum = 6 to 7 days) (Figure 10a). Based on the

data for equivalent incubation periods (5 days), the total volatile outputs of the E. carotovora treatments were, on the average, 112X greater than for the C. sepedonicum treatments, a difference significant at the 1% level (Table 10b). This reflects the differences in the rate of disease development. When the data for the entire 13 day C. sepedonicum incubation period were included in the analysis, there was no significant difference between the average total volatile outputs of the E. carotovora and C. sepedonicum treatments (Table 10b). This similarity might have been expected since it is the availability of metabolizable substrate that, in the end, determines the potential volatile output. In the C. sepedonicum treatments, the total daily volatile outputs declined towards the end of the incubation period (Figure 10a). This may have been due to the exhaustion of the metabolizable tuber tissues. The drop-off was not observed in the E. carotovora treatments likely because the excessive bacteria seepage limited the sampling period.

As in the E. carotovora trials, the total volatile production of the C. sepedonicum treatments (day 1 to 13) increased exponentially over the incubation period (Table 10e). However, the C. sepedonicum output data fit a parabolic regression equation more accurately than the linear equation that was found to most accurately suit the E. carotovora data. The parabolic equation better described the C. sepedonicum data because it allowed for the decline in volatile production towards the end of the test period.

As in the E. carotovora infections, the linear regression equation for the C. sepedonicum treatment had a significantly different slope and overall equation than the control treatments (Table 10f). Although the slope of the C. sepedonicum line was steeper than the control's indicating a more rapid increase in volatile production, the Y intercepts for the C. sepedonicum and control treatments were relatively similar (Figure 10a).

This again reflects the very slow development of the C. sepedonicum infection.

The slope and general equation of the regression lines for the C. sepedonicum data (days 1 to 5) were significantly different (P = 0.01) from the values calculated for the E. carotovora treatment (Table 10f). Based on the data for days 1 to 13, in the C. sepedonicum trial, the slopes for the regression lines of the two treatments remained significantly different (P = 0.05), however, the overall linear equations were not significantly different (P = 0.05). The Y intercept of the E. carotovora equation was greater than in the C. sepedonicum equation indicating a much earlier disease-induced increase in volatile production. The slope of the regression line was much steeper in the E. carotovora treatments. This reflected the much more rapid development of the soft rot infection.

The C. sepedonicum profiles featured a more limited range of volatiles than occurred in the E. carotovora treatments. Although there was considerable overlap in the compounds identified in the respective profiles (REFERENCE TABLE C), a single compound (RT = 15.6 min) identified as a major component in the C. sepedonicum profile ($\bar{X}$ PTPA value = 41%) was not detected in the E. carotovora profiles. Conversely, several compounds not isolated in the C. sepedonicum profiles were relatively major components of the E. carotovora profiles (i.e., ethanal, 1-propanal/2-propanone, and four unidentified compounds, RT = 7.1, 12.7, 14.1, and 16.3 min).

Since the diagnostic compounds tended to play relatively important roles in their respective profiles, the monitoring of their presence or absence in a volatile profile would seem to be a relatively valid technique for differentiating between the C. sepedonicum and E. carotovora profiles. However, three of the four compounds isolated in the E. carotovora profile, but not in the C. sepedonicum profile, were detected at significant levels

in the profiles of the non-inoculated control treatments. Whether a baseline metabolite would cease to be produced/released in detectable quantities due to the introduction of a certain pathogen is questionable. It is possible that the C. sepedonicum infection might cause some alterations in the metabolic processes of the potato resulting in changes in the baseline volatile profile. However, it seems more likely that these compounds would continue to be present in the C. sepedonicum infection, but were simply not detected in this trial.

As in the E. carotovora infections, the average number of peaks/chromatogram for the C. sepedonicum treatments tended to increase over the incubation period (Table 10c). Again, whether this change was due to changes in metabolic pattern over the incubation or simply due to the increase in the detectability of the various compounds as the overall volatile output increased was uncertain. Unlike the E. carotovora treatments, on a day to day basis the C. sepedonicum chromatograms were not significantly more complex than those of the controls (Table 10d). This again reflects the slow development of the ring-rot infection. Based on the C. sepedonicum data from days 1 to 5, the equivalent E. carotovora profiles were much more complex,again due to the differences in the rates of disease development (Table 10d). However, when the C. sepedonicum data from days 1 to 13 were included in the calculations, there was no significant difference between the average complexities of the E. carotovora and C. sepedonicum treatments. This again indicates some parallelism between the two decay producing pathogens that is not apparent in the various analyses of the relative rates of disease development.

The peak area and PTPA signatures for the C. sepedonicum treatment were quite distinct from the equivalent signatures of the E. carotovora and

control treatments (Table 10h). Although there was considerable overlap in the compounds involved in the signatures, the compound (X) unique to the C. sepedonicum profiles featured quite prominently in both the day 1 to 5 and days 1 to 13 C. sepedonicum signatures. Conversely, the various compounds unique to the E. carotovora and control profiles (B, D, H, and J) featured quite prominently in the respective signatures. Additionally, the order of the dominant peaks and the homologous peak groupings in the C. sepedonicum signatures were very different from the arrangements found in the E. carotovora and control signatures.

In comparing the mean peak areas ($LOG_{10}$) for the various metabolites in the C. sepedonicum (day 1 to 5) and control (day 1 to 5) profiles, the peaks were approximately equally divided, in terms of whether the control or the C. sepedonicum profile had featured the significantly greater quantity of the diagnostic compound (Table 10g). This was not an unexpected result, since through days 1 to 5 the total volatile outputs of the C. sepedonicum treatments were not very different from the controls. However, in the peak area comparisons of the C. sepedonicum (days 1 to 13) and control (days 1 to 13) profiles, the same equal division occurred, despite the fact that by the end of the incubation period the total volatile outputs of the C. sepedonicum treatments were 30 to 40X greater than the controls. It is clear from this that the increased volatile output that occurred during the progression of the C. sepedonicum was channelled through a limited number of compounds. This situation is in direct contrast to the results observed in the comparisons between the E. carotovora and control treatments. Once the E. carotovora infection had developed virtually every metabolite recorded in the control profile could be found at significantly greater levels in the E. carotovora profiles. The E. carotovora infection

appeared to produce a more general, non-specific increase in the normal baseline volatiles than occurred in the C. sepedonicum infection. In direct comparisons between the C. sepedonicum (day 1 to 5) and the E. carotovora peak area values, a number of metabolites were found to occur at higher levels in the E. carotovora profile (Table 10g). This again reflected the slow rate of development of the C. sepedonicum infection relative to E. carotovora. When the data for C. sepedonicum days 1 to 13 were included in the peak area analyses, a number of significant differences continued to exist between the C. sepedonicum and E. carotovora profiles, despite the fact that the total volatile outputs of the two treatments were approximately equal. The compounds occurring at diagnostically different levels were divided fairly evenly in terms of which of the treatments, C. sepedonicum or E. carotovora, had produced the significantly greater quantity. Although the two infections had developed volatile profiles that were parallel in terms of their total outputs and component range, the relative importance of these components was very different in the two profiles.

The relationship between the C. sepedonicum profile and the profiles for the E. carotovora and control treatments is perhaps best illustrated by comparisons of the PTPA values. At both incubation period intervals (C. sepedonicum days 1 to 5 and days 1 to 13), the list of compounds that occurred at significantly different relative levels in the C. sepedonicum and E. carotovora treatments was virtually identical to the list of compounds that had significantly different PTPA levels in the C. sepedonicum and control comparisons (Table 10i). In the pairwise comparisons with the C. sepedonicum profile components, the control and E. carotovora treatments were essentially interchangable in terms of the final result of the comparison. For instance, if in a C. sepedonicum versus E. carotovora comparison the PTPA

value of a compound was greater in the E. carotovora profile, then invariably in the C. sepedonicum versus control comparison, the control PTPA value for that compound would be significantly greater than in the C. sepedonicum profile.

In summary, a number of differences in volatile the production patterns of C. sepedonicum and E. carotovora were apparent, i.e. volatile production per unit time, chromatogram complexity, etc.

Unlike the situation in the test trials, in commercial storages tuber contamination does not occur simultaneously throughout a mass of potatoes nor are the environmental conditions ideally suited to the uniform development of the infection. Consequently, it is likely that any volatile production differences between two pathogens that were due to differences in the rates of disease development would be masked by the asynchronous and variable disease development that occurs in commercial storages. If the differences in the volatile production patterns of the C. sepedonicum and E. carotovora that stemmed from the different rates of disease development were ignored, many aspects of the volatile profiles, i.e. $\bar{X}$ volatile production, volatile production versus controls, and chromatogram complexity were relatively similar.

The volatile profiles of the two pathogens featured a number of commonly shared metabolites, but each pathogen produced one or more unique peaks, relative to the other infection. Perhaps most importantly, the relative ratios of the metabolites in the respective profiles were consistently different. Although more comparisons are needed, it is these types of stable differential factors that would have the greatest potential as means of distinguishing between the volatile profiles of E. carotovora and C. sepedonicum outbreaks in commercial storages.

# SUMMARY


## Headspace Sampling System

Direct sampling via a gas-tight syringe yielded accurate representative information on the composition of the headspace atmospheres tested, but was only useful in situations that allowed the accumulation of unrealistically elevated levels of the volatile metabolites. Under all operating conditions encountered in this study, the volatile absorbent Chromosorb 105 was found to be very effective as a means of trapping and concentrating the volatiles from larger samples of headspace. Utilizing an insert/liner as a trap produced a highly desirable one-step trapping/injection procedure. Flexible gas-tight bags proved to be superior to glass containers as both headspace confinement vessels and disease incubation chambers.

## Volatile Production

A number of quantitative and qualitative changes occurred in the volatile profiles of potatoes during the development of an E. carotovora infection. Elevated volatile production levels were detected very early in the development of the infection. Total volatile production increased exponentially as the disease spread. A wide range of low molecular weight organics were identified in the volatile profiles (aldehydes, ketones, and alcohols). The number of compounds detected in the volatile profiles tended to increase as the infections developed. Different relative amounts of the individual compounds were produced at various stages during the progression of the infection.

Total volatile production/unit time was the most dependable means of differentiating between healthy and diseased tubers on the basis of their volatile production characteristics. A number of the volatiles identified in the diseased profiles were also produced by healthy potatoes (both wounded and non-wounded) but, on the average, the profiles of the diseased treatments featured more peaks than the controls. Comparisons of the relative importance of the individual metabolites in the diseased and healthy profiles were difficult due to variable results.

The volatile production characteristics of E. carotovora var. carotovora and E. carotovora var. atroseptica infections were very similar when rearing conditions were suitable for the growth of both pathogens. Different strains of the E. carotovora bacteria produced similar volatile outputs, although small differences in the relative growth rates of the pathogens were reflected in their volatile production.

The volatile production characteristics of both healthy and E. carotovora infected 'Norland' and 'Russet Burbank' potatoes were very similar.

A number of factors which influenced the rate or pattern of disease development consequently influenced the volatile characteristics of the infections. Increasing the inoculum size hastened disease development, changing the rate of volatile production/unit time, but not the overall volatile production pattern. The rate of volatile production by both diseased and healthy treatments was strongly influenced by the incubation temperature. The range of metabolites recorded in the profiles was not affected by the temperature but the relative abundance of the various compound was to some extent temperature dependent. Temperature related differences in the rate of development of E. carotovora var. carotovora and E. carotovora var. atroseptica infections were reflected by quantitative and qualitative differences in their volatile profiles.

The volatile profiles of E. carotovora and C. sepedonicum infections differed in many respects. Total volatile production/unit time was much lower in the C. sepedonicum infection, which reflects the slower development of the pathogen. The C. sepedonicum profiles lacked a number of the compounds isolated in the E. carotovora profiles, but the C. sepedonicum infection also featured one unique diagnostic compound. The relative importance (PTPA values) of the individual peaks in the C. sepedonicum profiles were clearly different from the same peaks in the E. carotovora profiles.

# CONCLUSION

Total volatile production appeared to be the most consistent and reliable of the volatile production factors as an indicator of the development of an E. carotovora infection. The various other factors were either not disease specific (i.e. peak presence, peak number) or were excessively influenced by variability in disease development, temperature conditions, etc. (i.e. relative peak area). In commercial storages, two volatile monitoring strategies could be employed based on the total volatile production/concentration: 1) assuming that baseline volatile production/concentration levels could be determined for a 'healthy' storage the presence of any E. carotovora induced decay would be indicated by volatile production above baseline levels. However, determining a stable baseline would be difficult due to changes with the year, the load, the cultivar, the temperature, etc. Additionally, the volatiles produced by the E. carotovora infections could be confused with the elevated outputs produced by the wounding, handling, or sprout-inhibition treatment of the tubers. 2) The more dependable strategy involves the progressive monitoring of the volatile production of the potatoes throughout the storage period. The development of any E. carotovora infections would be detected by the resulting steady rise in the total volatile production/concentration in the storage. The previously mentioned causes of volatile production (wounding, sprout inhibition) would not produce this steady rise in volatile production.

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# APPENDICES

APPENDIX 2a. Analysis of variance for mean of total of peak areas[1)] averaged over the incubation period for two cultivars of potato treated with E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 2 | 12.961 | 6.481 | 498.538** |
| Cultivar (C) | 1 | 0.001 | 0.001 | 0.077N.S. |
| DXC | 2 | 0.111 | 0.056 | 4.308N.S. |
| Error | 8 | 0.105 | 0.013 | |
| Total | 13 | 13.178 | | |

**Significant at the 1% level.

N.S. Not significant at the 5% level.

1) Log of sum of all individual peak areas.

APPENDIX 2b. Analysis of variance for mean number of peaks/chromatogram averaged over the incubation period for two cultivars of potato with E. carotovora var. carotovora, E. carotovora var. atroseptica, and control treatments.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 2 | 40.042 | 20.021 | 22.345** |
| Cultivar (C) | 1 | 0.001 | 0.001 | 0.001N.S. |
| DXC | 2 | 1.865 | 0.932 | 1.040N.S. |
| Error | 8 | 7.165 | 0.896 | |
| Total | 13 | 49.073 | | |

**Differences significant at the 1% level.

N.S. Differences not significant at the 5% level.

APPENDIX 3a. Analysis of variance for means of total of peak areas[1)] averaged over the incubation period for two cultivars of potato inoculated with E. carotovora var. carotovora and E. carotovora var. atroseptica.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 1 | 1.403 | 1.403 | $1.271^{N.S.}$ |
| Cultivar(C) | 1 | 1.495 | 1.495 | $1.354^{N.S.}$ |
| DXC | 1 | .368 | .368 | $0.333^{N.S.}$ |
| Error | 4 | 4.416 | 1.104 | |
| Total | 7 | 7.682 | | |

N.S. Differences not significant at the 5% level.

1) Log of sum of all individual peak areas.

APPENDIX 3b. Analysis of variance for mean number of peaks/chromatogram for 'Russet Burbank' and 'Norland' tubers inoculated with E. carotovora var. carotovora or E. carotovora var. atroseptica.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 1 | 30.9 | 30.9 | $6.3^{N.S.}$ |
| Cultivar (C) | 1 | 2.6 | 2.6 | $0.5^{N.S.}$ |
| DXC | 1 | 0.3 | 0.3 | $0.1^{N.S.}$ |
| Error | 4 | 19.6 | 4.9 | |
| Total | 7 | | | |

N.S. Differences not significant at the 5% level.

APPENDIX 6a. Analysis of variance for means of total of peak areas[1)] averaged over the incubation period for two cultivars of potato inoculated with two strains of E. carotovora var. atroseptica and three strains of E. carotovora var. carotovora.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 4 | 10.583 | 2.636 | 47.07** |
| Cultivar (C) | 1 | 0.173 | 0.173 | 3.09N.S. |
| CXD | 4 | 0.583 | 0.146 | 2.61N.S. |
| Error | 10 | 0.557 | 0.056 | |
| Total | 19 | 11.897 | | |

** Significant at the 1% level.

N.S. Not significant at the 5% level.

1) Log of sum of all individual peak areas.

APPENDIX 6b. Mean number of peaks/chromatogram averaged over the incubation period for two cultivars of potato inoculated with two strains of E. carotovora var. atroseptica and three strains of E. carotovora var. carotovora.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 4 | 54.27 | 13.57 | 24.89** |
| Cultivar (C) | 1 | 1.75 | 1.75 | 3.22N.S. |
| CXD | 4 | 5.84 | 1.46 | 2.67N.S. |
| Error | 10 | 5.45 | 0.55 | |
| Total | 19 | 67.31 | | |

**Significant at the 1% level.

N.S. Not significant at the 5% level.

APPENDIX 7a. Analysis of variance means of total of peak areas[1)] averaged over the incubation period for a mixed E. carotovora infection held at three incubation temperatures.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease (D) | 1 | 3.379 | 3.379 | 33.79** |
| Temperature (T) | 2 | 1.504 | 0.752 | 7.52** |
| DXT | 2 | 0.118 | 0.059 | 0.59N.S. |
| Error | 11 | 1.103 | 0.100 | |
| Total | 16 | 6.104 | | |

** Significant at the 1% level.

N.S. Not significant at the 5% level.

1) Log of sum of all individual peak areas.

APPENDIX 7b. Analysis of variance for mean number of peaks/chromatogram averaged over the incubation period for a mixed E. carotovora infection held at three incubation temperatures.

| Source of variation | D.F. | S.S. | M.S. | F-Ratio |
|---|---|---|---|---|
| Disease | 1 | 17.43 | 17.43 | 91.7** |
| Temperature | 2 | 11.09 | 5.54 | 29.2** |
| DXT | 2 | 0.36 | 0.18 | 0.9 N.S. |
| Error | 11 | 2.08 | 0.19 | |
| Total | 16 | 30.96 | | |

** Significant at the 1% level.

N.S. Not significant at the 5% level.

## APPENDIX A. Relationship between temperature and the vapor pressures of organic compounds.

The vapor pressure of organic compounds at different temperatures may be derived from the equation:

$$\text{Log}_{10}P = (-0.2185\ A/K) + B$$

where:

P = Vapor pressure in torrs.

K = Temperature in degrees Kelvin.

A = Molar heat of vaporization in calories/gram mole.

B = A constant specific to the individual compounds.